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(71) Applicant (for all designated States except US): MENDEL BIOTECHNOLOGY, INC. [US/US]; 21375 Cabot Boulevard, Hayward, CA 94541 (US).

(71) Applicants and

(72) Inventors: RIECHMANN, Jose Luis [ES/US]; 115 Moss Avenue #308, Oakland, CA 94611 (US). REUBER, Lynne [US/US]; 2000 Walnut Avenue, Fremont, CA 94538 (US). KEDDIE, James [GB/US]; 54 McLellan Avenue, San Mateo, CA 94403 (US). RATCLIFFE, Oliver [GB/US]; 814 East 21st Street, Oakland, CA 94606 (US). HEARD, Jacqueline [US/US]; 810 Guildford Avenue, San Mateo, CA 94402 (US). SAMAHA, Raymond [US/US]; 2224 Albert Lane, Capitola, CA 95010 (US). YU, Guo-Liang

[CN/US]; 242 Gravatt Drive, Berkeley, CA 94705 (US). **JIANG, Cai-Zhong** [CN/US]; 34495 Heathrow Terrace, Fremont, CA 94555 (US).

(74) Common Representative: MENDEL BIOTECHNOL-OGY, INC.; Guerrero, Karen, 21375 Cabot Boulevard, Hayward, CA 94545 (US).

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(54) Title: PLANT DEVELOPMENTAL GENES

# PLANT DEVELOPMENTAL GENES

#### RELATED APPLICATION INFORMATION

The present invention claims the benefit from US Provisional Patent Application Serial

Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait Modification III" filed August 22, 2000.

#### FIELD OF THE INVENTION

This invention relates to the field of plant biology. More particularly, the present invention pertains to compositions and methods for phenotypically modifying a plant.

#### BACKGROUND OF THE INVENTION

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Transcription factors can modulate gene expression, either increasing or decreasing (inducing or repressing) the rate of transcription. This modulation results in differential levels of gene expression at various developmental stages, in different tissues and cell types, and in response to different exogenous (e.g., environmental) and endogenous stimuli throughout the life cycle of the organism.

Because transcription factors are key controlling elements of biological pathways, altering the expression levels of one or more transcription factors can change entire biological pathways in an organism. For example, manipulation of the levels of selected transcription factors may result in increased expression of economically useful proteins or metabolic chemicals in plants or to improve other agriculturally relevant characteristics. Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a plant's traits.

The present invention provides novel transcription factors useful for modifying a plant's phenotype in desirable ways, such as modifying a plant's structure or development.

# SUMMARY OF THE INVENTION

In a first aspect, the invention relates to a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-23, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-23, or a

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complementary nucleotide sequence thereof; (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's structure and development characteristics; (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; and (1) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-23. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above.

In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention is a plant lacking a nucleotide sequence encoding a polypeptide described above. The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

Furthermore, the invention relates to a method for producing a plant having modified structure and development traits. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for modified structure and development traits.

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In another aspect, the invention relates to a method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method comprises placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant structure and development trait.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant structure and development characteristics phenotype.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

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#### **DETAILED DESCRIPTION**

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's structure or development characteristics when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying the structure and size of flowers, leaves, roots, the plant as a whole, or the like, apical dominance, branching patterns, number of organs, organ identity, whether a plant is sterile or not, the vascularization of a plant, or the developmental staging of a plant, such as when senescence is triggered.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one or more of the following transcription factor families: the AP2 (APETALA2) domain transcription

factor family (Riechmann and Meyerowitz (1998) J. Biol. Chem. 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) Trends Genet. 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) J. Biol. Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-5 571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the 10 squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371–1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNAbinding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-15 binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e, expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

# **DEFINITIONS**

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A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The

polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

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A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polynucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for

the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell.

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The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by available

biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

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"Trait modification" refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution observed in wild type plant.

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Trait modifications of particular interest include those to seed ( such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenyllipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that can be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time, flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as

plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

#### POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

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The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's structure and development characteristics.

Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

Additional polynucleotides of the invention were identified by screening

15 Arabidopsis thaliana and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate

5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the structure and development characteristics of the plants were observed. Therefore, the polynucleotides and polypeptides can be employed to improve the structure and development characteristics of plants.

#### Making polynucleotides

The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-

coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

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Sambrook and Berger, all supra.

A variety of methods exist for producing the polynucleotides of the invention. Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, <u>Guide to Molecular Cloning Techniques, Methods in Enzymology</u> volume 152 Academic Press, Inc., San Diego, CA ("Berger"); Sambrook et al., <u>Molecular Cloning - A Laboratory Manual</u> (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and <u>Current Protocols in Molecular Biology</u>, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis). Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which

Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically ligated to produce a desired sequence, e.g., a polynucleotide encoding all or part of a

PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. *See*, e.g., Ausubel,

transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) <u>Tetrahedron Letters</u> 22:1859-69; and Matthes et al. (1984) <u>EMBO J.</u> 3:801-5. According to such methods, oligonucleotides are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

# **HOMOLOGOUS SEQUENCES**

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Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from Arabidopsis thaliana or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such pine, poplar and eucalyptus.

Transcription factors that are homologous to the listed sequences will typically share at least about 30% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide

sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

#### Identifying Nucleic Acids by Hybridization

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Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The T<sub>m</sub> is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization

of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

# SEQUENCE VARIATIONS

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It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

Table 1

Amino acids			Codon					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	TGC	TGT				
Aspartic acid	Asp	D	GAC	GAT				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	TTC	TTT				
Glycine	Gly	G	GGA	GGC	GGG	GGT		
Histidine	His	H	CAC	CAT				
Isoleucine	Ile	I	ATA	ATC	ATT			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT
Methionine	Met	M	ATG					
Asparagine	Asn	N	AAC	AAT				
Proline	Pro	P	CCA	CCC	CCG	CCT		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT
Threonine	Thr	T	ACA	ACC	ACG	ACT		
Valine	Val	V	GTA	GTC	GTG	GTT		
Tryptophan	Trp	W	TGG					
Tyrosine	Tyr	Y	TAC	TAT				

Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

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In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be

combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

10 <u>Table 2</u>

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Residue	Conservative Substitutions			
Ala	Ser			
Arg	Lys			
Asn	Gln; His			
Asp	Glu			
Gln	Asn			
Cys	Ser			
Glu	Asp			
Gly	Pro			
His	Asn; Gln			
Ile	Leu, Val			
Leu	Ile; Val			
Lys	Arg; Gln			
Met	Leu; Ile Met; Leu; Tyr			
Phe				
Ser	Thr; Gly			
Thr	Ser;Val			
Trp	Tyr			
Tyr	Trp; Phe			
Val	Ile; Leu			

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of

the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

# 10 <u>FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED</u> EVOLUTION

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In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) Nature 370:389-391, and Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and *E. coli* prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51; 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

# EXPRESSION AND MODIFICATION OF POLYPEPTIDES

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Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

# Vectors, Promoters and Expression Systems

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The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642, for dicotyledonous plants.

Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou (1991) Bio/Technology 9: 957-962) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technology 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48, and for Agrobacterium-mediated DNA transfer (Ishida et al. (1996) Nature Biotech 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or

developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which can be useful for expressing the TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (*see*, e.g., Odel et al. (1985) Nature 313:810); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

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A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known promoters have been characterized and can favorable be employed to promote expression of a polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No. 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flowerspecific (Kaiser et al, (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlemeier et al. (1989) Plant Cell 1:471, and the maize rbcS promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., wunI, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-

396, and the PDF1.2 promoter described in Manners et al. (1998) <u>Plant Mol. Biol.</u> 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) <u>Plant Mol Biol</u> 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) <u>Science</u> 270: 1986-1988); or late seed development (Odell et al. (1994) <u>Plant Physiol</u> 106:447-458).

Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

# Additional Expression Elements

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Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

#### **Expression Hosts**

The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments thereof) by recombinant techniques. Host cells are genetically engineered (i.e, nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, *etc.* The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of Agrobacterium tumefaciens or A. rhizogenes carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by Agrobacterium tumefaciens, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

# Modified Amino Acids

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The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-

Polypeptides of the invention may contain one or more modified amino acids.

translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

# **IDENTIFICATION OF ADDITIONAL FACTORS**

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A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phentoype or trait of interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g, a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al. (1999) Nature Biotechnology 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any

method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast Saccharomyces cerevisiae that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

# 20 IDENTIFICATION OF MODULATORS

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In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northerns, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) <u>Current Protocols in Molecular Biology</u>, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified

molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

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Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3):309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. Science (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al. Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

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Indeed, entire high throughput screening systems are commercially available. These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell,

plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

#### **SUBSEQUENCES**

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Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like, according to methods as noted *supra*.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides, or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

#### PRODUCTION OF TRANSGENIC PLANTS

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#### Modification of Traits

The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the seed characteristics of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, modified structure and development characteristics, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

#### Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997)

Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably,

the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

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Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by a transcription factor or transcription factor homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous transcription factor, thereby reducing or eliminating it's activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single transgene insertion

event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

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A plant trait can also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledenous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean,

clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcurbitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) <u>Handbook of Plant Cell Culture – Crop Species</u>. Macmillan Publ. Co. Shimamoto et al. (1989) <u>Nature</u> 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

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Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumeficiens* mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

#### INTEGRATED SYSTEMS—SEQUENCE IDENTITY

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Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may be used to associate or link certain functional benefits, such modified structure and development characteristics, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madision, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., supra.

A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. <u>J. Mol. Biol</u> 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is

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referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

The integrated system, or computer typically includes a user input interface allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular

phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

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Thus, the invention provides methods for identifying a sequence similar or homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

Any sequence herein can be entered into the database, before or after querying the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

# **EXAMPLES**

The following examples are intended to illustrate but not limit the present invention.

# 25 EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of –4 or –5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, Arabidopsis *thaliana* cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60°C) and labeled with <sup>32</sup>P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO<sub>4</sub> pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSCC, 1% SDS at 60°C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the Marathon<sup>TM</sup> cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the Marathon<sup>TM</sup> Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

# EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

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The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with SalI and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into

competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

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Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

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After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of *Agrobacterium tumefaciens* cells for transformation were made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. *Agrobacterium* strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance ( $A_{600}$ ) of 0.5-1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250  $\mu$ l chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125  $\mu$ l chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100  $\mu$ l and 750  $\mu$ l, respectively. Resuspended cells were then distributed into 40  $\mu$ l aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 μl of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 μF and 200 μF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 μg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

# 30 <u>EXAMPLE IV. TRANSFORMATION OF ARABIDOPSIS PLANTS WITH AGROBACTERIUM TUMEFACIENS WITH EXPRESSION VECTOR</u>

After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to

transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28°C with shaking for 2 days until an absorbance (A<sub>600</sub>) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 μM benzylamino purine (Sigma), 200 μl/L Silwet L-77 (Lehle Seeds) until an absorbance (A<sub>600</sub>) of 0.8 was reached.

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Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75  $\mu$ E/m²/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

### 20 EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H<sub>2</sub>O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H<sub>2</sub>O. The seeds were stored in the last wash water at 4° C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75 μE/m²/sec) at 22-23°

C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants ( $T_1$  generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

Primary transformants were crossed and progeny seeds  $(T_2)$  collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

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# EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

The screening of insertion mutagenized *Arabidopsis* collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) Plant Cell 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 pb to each others, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

# 25 EXAMPLE VII. IDENTIFICATION OF STRUCTURE AND DEVELOPMENT CHARACTERISTICS PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT PLANTS

Experiments were performed to identify those transformants or knockouts that exhibited a modified structure and development characteristics. For such studies, the transformants were observed by eye to identify novel structural or developmental characteristics associated with the ectopic expression of the polynucleotides or polypeptides of the invention.

Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

Table 3

SEQ ID No.	GID		Phenotype observed
		overexpressor (KO)	
1	G727	OE	Plants were small, and more dark green in
			color, late flowering and poorly fertile.
3	G732	OE	Plants were small and inflorescence was
			unelongated. Flowers parts appeared to be un-
			elongated and the plants were semi-sterile.
5	G9	OE	Increased root mass
7	G428	OE	Lobed and highly serrated leaves and
			abnormal first and second whorl floral organs
9	G869	OE	Undeveloped or small anthers
11	G1269	OE	Extended petioles and leaves pointed upwards
13	G1038	OE	Altered leaf shape
15	G438	KO	Reduced lignin in stem
17	G571	KO	Delayed senescence at the end of the plant
			lifecycle
19	G748	OE	More vascular bundles in stem
21	G431	OE	Severe developmental abnormalities such as
			altered branching, twisted rosette leaves,
			flowers with missing pistils, fused stamens and
			atypical numbers of petals and stamens,
			reduced secondary bolts, and lack of cauline
			leaves.
23	G187	OE	Plants had long, thin cotyledons and reduced
			apical dominance. Several flower
			abnormalities, including underdeveloped,
			sepaloid petals and underdeveloped anthers
	C 170		were also observed.
25	G470	OE	Plants were sterile due to failure of anthers to
	0.61.5		elongate
27	G615	OE	Plants were sterile due to failure of anthers to
			develop and failure of stamens to elongate.
			Fused cotyledons and absence of a shoot apical
20	G1070	0.5	meristem and true leaves was also observed.
29	G1073	OE	Increased plant size and serrated leaves

For a particular overexpressor that shows a less beneficial structure and development characteristic, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows a less beneficial structure and development characteristic, it may be more useful to select a plant with an increased expression of the particular transcription factor.

#### EXAMPLE VIII. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

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Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) <u>J. Mol. Biol.</u> 215:403-410; and Altschul et al. (1997) <u>Nucl. Acid Res.</u> 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) <u>Proc. Natl. Acad. Sci. USA</u> 89: 10915-10919).

Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-54 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-54, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6e-40 is 3.6 x 10<sup>-40</sup>. For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-Arabidopsis genes shown in Figure 3 and the Arabidopsis genes in the sequence listing are: SEQ ID No. 1: 36%-69%; SEQ ID No. 3: 46%-54%; SEQ ID No. 5: 57%-72%; SEQ ID No. 7: 54%-69%; SEQ ID No. 9: 31%-68%; SEQ ID No. 11: 47%-90%; SEQ ID No. 13: 34%-82%; SEQ ID No. 15: 49%-88%; SEQ ID No. 17: 56%-67%; SEQ ID No. 19: 39%-61%; SEQ ID No. 21: 61%-87%; SEQ ID No. 23: 38%-85%; SEQ ID No. 25: 44%-94%; SEQ ID No. 27: 35%-44%; SEQ ID No. 29: 37%-71%; SEQ ID No. 31: 38%-77%; SEQ ID No. 33: 57%-69%; SEQ ID No. 35: 54%-69%; SEQ ID No. 37: 60%-75%; SEQ ID No. 39: 47%-65%; SEQ ID No. 41: 60%-88%; SEQ ID No. 43: 43%-87%; and SEQ ID No. 45: 53%-97%.

The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the structure and development characteristics of a plant.

All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

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1. A transgenic plant with modified structure and development characteristics, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-23, or a complementary nucleotide sequence thereof;
  - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
  - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-23, or a complementary nucleotide sequence thereof;
  - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
  - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
  - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
  - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's structure and development characteristics;
  - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
  - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
  - (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23;
- (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; and
  - (l) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-23.
- The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissue-active promoter operably linked to said nucleotide sequence.
  - 3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf,

banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

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- 4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
  - (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-23, or a complementary nucleotide sequence thereof;
  - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
    - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-23, or a complementary nucleotide sequence thereof;
    - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
    - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
    - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
    - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's structure and development characteristics;
    - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
    - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
    - (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23;
    - (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; and
- (1) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-23.

5. The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.

- 6. A cloning or expression vector comprising the isolated or recombinant polynucleotide of claim 4.
  - 7. A cell comprising the cloning or expression vector of claim 6.
  - 8. A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.

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- 9. A composition produced by one or more of:
  - (a) incubating one or more polynucleotide of claim 4 with a nuclease;
  - (b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;
  - (c) incubating one or more polynucleotide of claim 4 with a polymerase;
  - (d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;
  - (e) incubating one or more polynucleotide of claim 4 with a cloning vector, or
  - (f) incubating one or more polynucleotide of claim 4 with a cell.
- 10. A composition comprising two or more different polynucleotides of claim 4.

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- 11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.
- 12. A plant ectopically expressing an isolated polypeptide of claim 11.

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- 13. A method for producing a plant having a modified structure and development characteristic, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for modified structure and development characteristics thereby providing the modified plant with a modified structure and development characteristics.
- 14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.

15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:

- (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

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- 16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.
- 10 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.
  - 18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:
    - (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,
    - (b) monitoring one or more of:
      - (i) expression level of the polynucleotide in the plant;
      - (ii) expression level of the polypeptide in the plant;
      - (iii) modulation of an activity of the polypeptide in the plant; or
      - (iv) modulation of an activity of the polynucleotide in the plant.
  - 19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.
  - 20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant structure and development characteristics phenotype.

- 21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:
  - (a) providing a sequence database; and,

(b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

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- 22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.
- 10 23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.
- The method of claim 21, further comprising linking the one or more of the
   polynucleotides of claim 4, or encoded polypeptides, to a modified plant structure and development characteristics phenotype.
  - 25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.

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- 26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.
- 27. A plant lacking a nucleotide sequence encoding a polypeptide of claim 11.

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G727	cDNA	
2	G727	protein	226-269
3	G732	cDNA	
4	G732	protein	31-9
5	G9	cDNA	
6	G9	protein	62-127
7	G428	cDNA	
8	G428	protein	229-292
9	G869	cDNA	
10	G869	protein	109-177
11	G1269	cDNA	
12	G1269	protein	27-83
13	G1038	cDNA	
14	G1038	protein	198-247
15	G438	cDNA	
16	G438	protein	22-85
17	G571	cDNA	
18	G571	protein	160-220
19	G748	cDNA	
20	G748	protein	112-140
21	G431	cDNA	
22	G431	protein	286-335
23	G187	cDNA	
24	G187	protein	172-228
25	G470	cDNA	-
26	G470	protein	61-393
27	G615	cDNA	
28	G615	protein	88-147
29	G1073	cDNA	
30	G1073	protein	33-42, 78-175

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
31	G1493	homolog of G727	cDNA	
32	G1493	homolog of G727	protein	242-289
33	G993	homolog of G9	cDNA	
34	G993	homolog of G9	protein	69-134
35	G867	homolog of G9	cDNA	
36	G867	homolog of G9	protein	59-124
37	G1930	homolog of G9	cDNA	
38	G1930	homolog of G9	protein	59-124
39	G1594	homolog of G428	cDNA	
40	G1594	homolog of G428	protein	262-325
41	G391	homolog of G438	cDNA	
42	G391	homolog of G438	protein	25-85
43	G390	homolog of G438	cDNA	
44	G390	homolog of G438	protein	18-81
45	G1548	homolog of G438	cDNA	
46	G1548	homolog of G438	protein	17-77

Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G727	7283684	2.20E-56	Glycine max
1	G727	7206180	8.40E-42	Medicago truncatula
11	G727	7614196	2.20E-40	Lotus japonicus
1	G727	572293	1.20E-31	Oryza sativa
1	G727	7218448	7.70E-30	Sorghum bicolor
1	G727	9291284	1.80E-27	Lycopersicon hirsutum
1	G727	8901641	5.10E-27	Hordeum vulgare
1	G727	8380453	6.60E-24	Gossypium arboreum
1	G727	9962201	2.10E-12	Cryptomeria japonica
1	G727	8122498	3.10E-08	Lycopersicon esculentum
3	G732	5048074	5.60E-30	Gossypium hirsutum
3	G732	4384142	6.10E-30	Lycopersicon esculentum
3	G732	7623218	6.10E-30	Gossypium arboreum
3	G732	4457220	1.80E-29	Capsicum chinense
3	G732	7284989	4.50E-28	Glycine max
3	G732	9650827	1.20E-27	Petroselinum crispum
3	G732	7205618	2.20E-26	Medicago truncatula
3	G732	3854258	1.40E-22	Populus tremula x Populus tremuloides
5	G9	7643366	6.80E-56	Medicago truncatula
5	G9	8669779		Glycine max
5	G9	8329389		Mesembryanthemum crystallinum
5	G9	9851335		Sorghum bicolor
5	G9	7412012	1.50E-41	Lycopersicon esculentum
5	G9	10450225		Solanum tuberosum
5	G9	8902194		Hordeum vulgare
5	G9	7722547		Lotus japonicus
5	G9	9696857		Triticum aestivum
5	G9	7324245		Lycopersicon pennellii
7	G428	3327268	5.50E-65	Ipomoea nil
7	G428	4589883		Nicotiana tabacum
7	G428	1814233		Solanum tuberosum
7	G428	7581978		Dendrobium grex Madame Thong-In
7	G428	4098241		Lycopersicon esculentum
<del></del>	G428	4099825		Picea mariana
7	G428	3462611		Pisum sativum
7	G428	3928842		Picea abies
<del></del> 7	G428	9699343		Triticum aestivum
7	G428	1008878		Zea mays
9	G869	10235055		Glycine max
9	G869	2213784		Lycopersicon esculentum
9	G869	3065894		Nicotiana tabacum
9	G869	8570080		Oryza sativa
9	G869	7560260		Medicago truncatula
9	G869	9850452		Sorghum bicolor
9	G869	9963144	1.10E-13	Cryptomeria japonica
9	G869	9660634	1.90E-13	Secale cereale
9	G869	9362061		Triticum aestivum
9	G869	7788764		Lotus japonicus
	1			Glycine max
11	G1269	9565366	7.00E-37	Lycopersicon esculentum
11	G1269	5272360	8.10E-37	
11	G1269			Medicago truncatula
11	G1269	9852711	2.10E-22	Sorghum bicolor

Figure 3B

050 15 11	CID	O b l AUD	Darahas	Onnaire
SEQ ID No.	GID	Genbank NID		Species
11	G1269	9255178		Zea mays
11	G1269	10447957	8.60E-15	
11	G1269	9435251		Hordeum vulgare
11	G1269	3858030	3.20E-09	Populus balsamifera subsp. trichocarpa
11	G1269	9696112	3.80E-09	Triticum aestivum
11	G1269	8213273	4.90E-09	Oryza sativa
13	G1038	8748344	8.00E-37	Medicago truncatula
13	G1038	7283684	5.20E-36	Glycine max
13	G1038	7218448	8.80E-36	Sorghum bicolor
13	G1038	572293	3.30E-35	Oryza sativa
13	G1038	8901641		Hordeum vulgare
13	G1038	9962201	2.20E-16	
13	G1038	7614196		Lotus japonicus
13	G1038	9291272		Lycopersicon hirsutum
13	G1038	8122498	0.0005	Lycopersicon esculentum
13	G1038	9883662	0.68	Triticum aestivum
15	G438	7209474		Oryza sativa
15	G438	7209911		Physcomitrella patens
15	G438	7571387		Medicago truncatula
15	G438	8330425	3.00E-66	Mesembryanthemum crystallinum
15	G438	6531152	1.60E-64	Lycopersicon esculentum
15	G438	6726825	4.70E-61	Glycine max
15	G438	5269007	7.00E-54	Zea mays
15	G438	9253000	1.70E-47	Solanum tuberosum
15	G438	8967371	4.40E-46	Hordeum vulgare
15	G438	2963336	1.60E-34	Pinus taeda
17	G571	6288681	1.50E-70	Nicotiana tabacum
17	G571	297019	1.60E-68	Zea mays
17	G571	10423526	2.20E-61	Oryza sativa
17	G571	5926681	4.20E-61	Triticum aestivum
17	G571	4959969	1.90E-59	Lycopersicon esculentum
17	G571	1372965	1.20E-56	Vicia faba
17	G571	8098832	1.20E-46	Hordeum vulgare
17	G571	9566058	2.00E-43	Glycine max
17	G571	765198	1.50E-41	Solanum tuberosum
17	G571	19679	3.80E-41	Nicotiana sp.
19	G748	853689	7.00E-87	Cucurbita maxima
19	G748	7242897	3.90E-59	Oryza sativa
19	G748	5888560	1.20E-45	
19	G748	6341666	5.60E-38	Glycine max
19	G748	10700058		Medicago truncatula
19	G748	7535776	5.00E-33	Sorghum bicolor
19	G748	9419494	2.10E-31	Hordeum vulgare
19	G748	9410157	1.00E-28	Triticum aestivum
19	G748	3929324	4.30E-25	Dendrobium grex Madame Thong-IN
19	G748	10449922	2.30E-23	
21	G431	7340349		Brassica oleracea
21	G431	3462611		Pisum sativum
21	G431	310568		Glycine max
21	G431	2251078		Nicotiana tabacum
21	G431	4098239		Lycopersicon esculentum
21	G431	1008878		Zea mays
21	G431	6942299	7.90E-62	Triticum aestivum

Figure 3C

OFO ID N	CID	Carbank NID	Dualua	Charies
SEQ ID No.	GID	Genbank NID	P-value	Species
21	G431	3327239	1.90E-61	Oryza sativa
21	G431	3928842		Picea abies
21	G431	2522483		Hordeum vulgare
23	G187	9304207		Sorghum bicolor
23	G187	9444636		Triticum aestivum
23	G187	5058292		Glycine max
23	G187	7721184		Lotus japonicus
23	G187	7562279		Medicago truncatula
23	G187	8105974		Lycopersicon esculentum
23	G187	9049477		Oryza sativa
23	G187	9187621		Solanum tuberosum
23	G187	5268376		Zea mays
23	G187	4894964		Avena sativa
25	G470	6917173		Lycopersicon pennellii
25	G470	8827792		Glycine max
25	G470	5272309		Lycopersicon esculentum
25	G470	7563870		Medicago truncatula
25	G470	5296108		Zea mays
25	G470	7339690	7.40E-57	Oryza sativa
25	G470	5047367	1.30E-51	Gossypium hirsutum
25	G470	9856054	9.70E-50	Sorghum bicolor
25	G470	3857884	1.10E-38	Populus balsamifera subsp. trichocarpa
25	G470	8174666	6.40E-37	Hordeum vulgare
27	G615	5566284	2.00E-28	Linaria vulgaris
27	G615	6358617	3.20E-27	Antirrhinum graniticum
27	G615	6358613	1.40E-26	Antirrhinum majus subsp. cirrhigerum
27	G615	6358545	8.60E-26	Digitalis purpurea
27	G615	6358538	1.40E-25	Antirrhinum braun-blanquetii
27	G615	6358541	1.40E-25	Misopates orontium
27	G615	6358542	1.40E-25	Antirrhinum molle
27	G615	6358573	1.40E-25	Misopates calycinum
27	G615	6358546	1.80E-25	Antirrhinum siculum
27	G615	2826867	2.70E-25	Antirrhinum majus
29	G1073	7238733	2.70E-55	Medicago truncatula
29	G1073	10843924	1.50E-44	Glycine max
29	G1073	7615218		Lotus japonicus
29	G1073			Lycopersicon esculentum
29	G1073			Pinus taeda
29	G1073		4.30E-25	
29	G1073	9252370	2.80E-24	Solanum tuberosum
29	G1073	5042437	5.80E-21	Oryza sativa
29	G1073	7536402	6.70E-20	Sorghum bicolor
29	G1073	9662742	2.70E-19	Secale cereale
31	G1493	7614196	2.20E-50	Lotus japonicus
31	G1493	9986889	6.10E-48	Glycine max
31	G1493	8748344	2.20E-38	Medicago truncatula
31	G1493	572293	1.70E-37	Oryza sativa
31	G1493	7218448	5.70E-33	Sorghum bicolor
31	G1493	9291284	9.70E-32	Lycopersicon hirsutum
31	G1493	8380453	1.60E-30	Gossypium arboreum
31	G1493		1.70E-30	Hordeum vulgare
31	G1493		6.90E-17	Cryptomeria japonica
31	G1493		1.50E-08	Lycopersicon esculentum
J 31	01433	0122430	1.001-00	Lyooporaloon cacalontum

Figure 3D

SEQ ID No.	GID	Genbank NID	P-value	Species
33	G993	7643366	1.20E-58	Medicago truncatula
33	G993	8329389		Mesembryanthemum crystallinum
33	G993	8669779		Glycine max
33	G993	9851335	6.30E-43	
33	G993	4384549		Lycopersicon esculentum
33	G993	10450225		Solanum tuberosum
33	G993	8902194		Hordeum vulgare
33	G993	7719409		Lotus japonicus
33	G993	8749037		Citrus x paradisi
33	G993	9247126		Oryza sativa
35	G867	7643366		Medicago truncatula
35	G867	8329389		Mesembryanthemum crystallinum
35	G867	8669779	2.70E-46	
35		10450225	3.60E-41	Solanum tuberosum
	G867			
35 35	G867	9851335		Sorghum bicolor Lycopersicon esculentum
	G867	9430646		
35	G867	8902194		Hordeum vulgare
35	G867	7722547		Lotus japonicus
35	G867	7324245		Lycopersicon pennellii
35	G867	8749037		Citrus x paradisi
37	G1930	7643366		Medicago truncatula
37	G1930	8329389		Mesembryanthemum crystallinum
37	G1930	6069592		Glycine max
37	G1930	10450225		Solanum tuberosum
37	G1930	9430646		Lycopersicon esculentum
37	G1930	9851335		Sorghum bicolor
37	G1930	7722547		Lotus japonicus
37	G1930	7324245		Lycopersicon pennellii
37	G1930	8902194		Hordeum vulgare
37	G1930	9697984		Triticum aestivum
39	G1594	3327268		Ipomoea nil
39	G1594	7581978		Dendrobium grex Madame Thong-In
39	G1594	4887609		Oryza sativa
39	G1594	1814233		Solanum tuberosum
39	G1594	4589883		Nicotiana tabacum
39	G1594	4098241		Lycopersicon esculentum
39	G1594	3928842		Picea abies
39	G1594	4099825		Picea mariana
39	G1594	4240538		Zea mays
39	G1594	1946219		Malus domestica
41	G391	7209474	4.70E-194	Oryza sativa
41	G391	7209911	2.10E-145	Physcomitrella patens
41	G391	7560927	8.70E-67	Medicago truncatula
41	G391	10808354	1.50E-61	Solanum tuberosum
41	G391	5893826		Lycopersicon esculentum
41	G391	8330425	8.60E-59	Mesembryanthemum crystallinum
41	G391	8284059	8.70E-57	Glycine max
41	G391	5269007	8.10E-46	Zea mays
41	G391	9419425	1.70E-43	Hordeum vulgare
41	G391	2963336	2.10E-37	Pinus taeda
43	G390	7209474		Oryza sativa
		7209911		Physcomitrella patens
43	G390	7209911		

# Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
43	G390	7409018	1.80E-68	Lycopersicon esculentum
43	G390	8071613	3.00E-60	Solanum tuberosum
43	G390	9466042	1.60E-59	Hordeum vulgare
43	G390	8284059	1.00E-57	Glycine max
43	G390	8330425	2.60E-44	Mesembryanthemum crystallinum
43	G390	5269007	4.60E-44	Zea mays
43	G390	2963336	4.90E-43	Pinus taeda
45	G1548	7209474	5.90E-169	Oryza sativa
45	G1548	7209911	3.30E-140	Physcomitrella patens
45	G1548	9253000	1.60E-76	Solanum tuberosum
45	G1548	9820423	1.40E-67	Glycine max
45	G1548	7570825	8.40E-67	Medicago truncatula
45	G1548	9456848	2.70E-55	Lycopersicon esculentum
45	G1548	9419425	1.40E-47	Hordeum vulgare
45	G1548	6626571	3.50E-46	Zea mays
45	G1548	8330425	4.20E-46	Mesembryanthemum crystallinum
45	G1548	3853847	2.70E-42	Populus tremula x Populus tremuloides

# MBI0018 Sequence Listing.ST25 SEQUENCE LISTING

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## MBI0018 Sequence Listing.ST25

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Val Asp Tyr Leu Ile Lys Pro Val Arg Ile Glu Ala Leu Lys Asn Ile 130 135 140

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Gly Asn Gly Arg Ser Ser Arg Lys Arg Lys Glu Glu Val Asp Asp 195 200 205

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Val Trp Ser Val Glu Leu His Gln Gln Phe Val Ala Ala Val Asn Gln 225 230 235

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Arg Arg Leu Gly Gly Val Ser Gln His Gln Gly Asn Met Asn His Ser 275 280 285

Phe Met Thr Gly Gln Asp Gln Ser Phe Gly Pro Leu Ser Ser Leu Asn 290 295 300

Gly Phe Asp Leu Gln Ser Leu Ala Val Thr Gly Gln Leu Pro Pro Gln 305 310 315 320

Ser Leu Ala Gln Leu Gln Ala Ala Gly Leu Gly Arg Pro Thr Leu Ala 325 330 335

Lys Pro Gly Met Ser Val Ser Pro Leu Val Asp Gln Arg Ser Ile Phe 340 345 350

#### MBI0018 Sequence Listing.ST25

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Val Ala Asp Gln Leu Pro Arg Gly Gly Pro Ser Met Leu Pro Ser Leu
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Gly Gln Gln Pro Ile Leu Ser Ser Ser Val Ser Arg Arg Ser Asp Leu 420 425 430

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Asn Gln Asp Ala Ala Thr Ala Thr Ala Thr Ala Ala Phe Ser Thr Ser 545 550 555

Glu Ala Tyr Ser Ser Ser Ser Thr Gln Arg Lys Arg Arg Glu Thr Asp 565 570 575

Ala Thr Val Val Gly Glu His Gly Gln Asn Leu Gln Ser Pro Ser Arg 580 585 590

Asn Leu Tyr His Leu Asn His Val Phe Met Asp Gly Gly Ser Val Arg 595 600 605

Val Lys Ser Glu Arg Val Ala Glu Thr Val Thr Cys Pro Pro Ala Asn 610 615 620

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Tyr Glu Lys His Gln Arg Val Trp Leu Gly Thr Phe Asn Glu Gln Glu 85 90 95

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Lys Leu Glu Phe Ser Lys Lys Lys Lys Gly Lys Leu Pro Arg Glu 225 230 235

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MBI0018 Sequence Listing.ST25

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Glu Ala Leu Lys Leu Tyr Gly Arg Ala Trp Arg Arg Ile Glu Glu His
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Val Gly Thr Lys Thr Ala Val Gln Ile Arg Ser His Ala Gln Lys Phe 65 70 75 80

Ile Pro Pro Pro Arg Pro Lys Arg Lys Pro Met His Pro Tyr Pro Arg 100 105 110

Lys Leu Val Ile Pro Asp Ala Lys Glu Met Val Tyr Ala Glu Leu Thr  $115 \,$  120  $\,$  125

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MBI0018 Sequence Listing.ST25 245 250 255

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cca Pro 385	tct Ser	ctc Leu	atg Met	gag Glu	gag Glu 390	gaa Glu	atg Met	aag Lys	cct Pro	cct Pro 395	tat Tyr	gag Glu	aca Thr	cca Pro	gca Ala 400	1439
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#### MBI0018 Sequence Listing.ST25

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Asn Met Pro Asp Met Asp Gly Phe Lys Leu Leu Glu His Val Gly Leu 65 70 75 80

Glu Leu Asp Leu Pro Val Ile Met Met Ser Val Asp Gly Glu Thr Ser 85 90 95

Arg Val Met Lys Gly Val His Thr Gly Ala Cys Asp Tyr Leu Leu Lys 100 105 110

Pro Ile Arg Met Lys Glu Leu Lys Ile Ile Trp Gln His Val Leu Arg

Lys Lys Leu Gln Glu Val Arg Asp Ile Glu Gly Cys Gly Tyr Glu Gly

Gly Ala Asp Trp Ile Thr Arg Tyr Asp Glu Ala His Phe Leu Gly Gly 145 150 160

Gly Glu Asp Val Ser Phe Gly Lys Lys Arg Lys Asp Phe Asp Phe Glu 165 170 175

Lys Lys Leu Leu Gln Asp Glu Ser Asp Pro Ser Ser Ser Ser Lys 180 185 190

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# MBI0018 Sequence Listing.ST25

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Ile	Pro	Thr	Val 420	Thr	Leu	Gln	Asp	Leu 425	Asp	Pro	Ser	Ala	Met 430	Lys	Leu	
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MBI0018 Sequence Listing.ST25

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agc gac Ser Asp 720														2389
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Glu Ala Trp Ser Val Pro Asp Val Leu Arg Pro Leu Tyr Glu Ser Ser 340 345 350

Lys Val Val Ala Gln Lys Met Thr Ile Ser Ala Leu Arg Tyr Ile Arg 355 360 365

Gln Leu Ala Gln Glu Ser Asn Gly Glu Val Val Tyr Gly Leu Gly Arg 370 375 380

Gln Pro Ala Val Leu Arg Thr Phe Ser Gln Arg Leu Ser Arg Gly Phe 385 395 400

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Leu Asn Asn Ile Ser Asn Ser Leu Ser Phe Leu Gly Gly Val Leu Cys 435 440 445

Ala Lys Ala Ser Met Leu Leu Gln Asn Val Pro Pro Ala Val Leu Ile 450 455 460

Arg Phe Leu Arg Glu His Arg Ser Glu Trp Ala Asp Phe Asn Val Asp 465 470 475 480

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490
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Leu Gln Ile Cys Thr Gly Ile Asp Glu Asn Ala Val Gly Ala Cys Ser 545 550 560

Glu Leu Ile Phe Ala Pro Ile Asn Glu Met Phe Pro Asp Asp Ala Pro 565 570 575

Leu Val Pro Ser Gly Phe Arg Val Ile Pro Val Asp Ala Lys Thr Gly 580 585 590

Asp Val Gln Asp Leu Leu Thr Ala Asn His Arg Thr Leu Asp Leu Thr 595 600 605

Ser Ser Leu Glu Val Gly Pro Ser Pro Glu Asn Ala Ser Gly Asn Ser 610 615 620

### MBI0018 Sequence Listing.ST25

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Tyr	Val	Arg	Ser 660	Val	Ile	Ser	Ser	Val 665	Gln	Arg	Val	Ala	Met 670	Ala	Ile	
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His 705	His	Leu	Gly	Ser	Glu 710	Leu	Leu	Thr	Ile	Asp 715	Ser	Leu	Gly	Ser	Asp 720	
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Cys	Ser	Leu	Lys 740	Pro	Gln	Pro	Val	Phe 745	Met	Phe	Ala	Asn	Gln 750	Ala	Gly	
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			cat gga aac ttc at His Gly Asn Phe Me 25	et.
			cca tca ctc ttc ct Pro Ser Leu Phe Le 40	
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Thr Gln Asn Leu Ala Met Arg Pro Pro Thr Ser Thr Leu Asn Ile Phe 65 70 75 80

Pro Ser Gln Pro Met His Ile Glu Pro Pro Pro Ser Ser Thr His Asn 85 90 95

Thr Asp Asn Thr Arg Leu Val Pro Ala Ala Gln Pro Ser Gly Ser Thr 100 105 110

Arg Pro Ala Ser Asp Pro Ser Met Asp Leu Thr Asn His Ser Gln Phe 115 120 125

His Gln Pro Pro Gln Gly Ser Lys Ser Ile Lys Lys Glu Gly Asn Arg 130 135 140

Lys Gly Leu Ala Ser Ser Asp His Asp Ile Pro Lys Ser Ser Asp Pro 145 150 155

Lys Thr Leu Arg Arg Leu Ala Gln Asn Arg Glu Ala Ala Arg Lys Ser 165 170 175

Arg Leu Arg Lys Lys Ala Tyr Val Gln Gln Leu Glu Ser Cys Arg Ile 180 185 190

Lys Leu Thr Gln Leu Glu Gln Glu Ile Gln Arg Ala Arg Ser Gln Gly 195 200 205

Val Phe Phe Gly Gly Ser Leu Ile Gly Gly Asp Gln Gln Gln Gly Gly

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Met Glu Tyr Ala Arg Trp Leu Glu Glu Gln Gln Arg Leu Leu Asn Glu
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Leu Arg Val Ala Thr Gln Glu His Leu Ser Glu Asn Glu Leu Arg Met 260 265 270

Phe Val Asp Thr Cys Leu Ala His Tyr Asp His Leu Ile Asn Leu Lys 275 280 285

Ala Met Val Ala Lys Thr Asp Val Phe His Leu Ile Ser Gly Ala Trp
290 295 300

Lys Thr Pro Ala Glu Arg Cys Phe Leu Trp Met Gly Gly Phe Arg Pro 305 310 315

Ser Glu Ile Ile Lys Val Ile Val Asn Gln Ile Glu Pro Leu Thr Glu 325 330 335

Gln Gln Ile Val Gly Ile Cys Gly Leu Gln Gln Ser Thr Gln Glu Ala 340 345 350

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Ser Arg Ser His Asn Phe His Glu Gln Ile

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Thr Pro Glu Leu Ser Asp Lys Asn Asn Asn Asn Cys Asn Asp Asn Ser 50 55 60

Phe Asn Asn Ser Lys Pro Glu Thr Leu Asp Lys Glu Glu Ala Thr Ser 65 70 75 80

Thr Asp Gln Ile Glu Ser Ser Asp Thr Pro Glu Asp Asn Gln Gln Thr 85 90 95

Thr Pro Asp Gly Lys Thr Leu Lys Lys Pro Thr Lys Ile Leu Pro Cys
100 105 110

Pro Arg Cys Lys Ser Met Glu Thr Lys Phe Cys Tyr Tyr Asn Asn Tyr 115 120 125

Asn Ile Asn Gln Pro Arg His Phe Cys Lys Ala Cys Gln Arg Tyr Trp 130 140

Thr Ala Gly Gly Thr Met Arg Asn Val Pro Val Gly Ala Gly Arg Arg 145 150 155

Lys Asn Lys Ser Ser Ser Ser His Tyr Arg His Ile Thr Ile Ser Glu 165 170 175

Ala Leu Glu Ala Ala Arg Leu Asp Pro Gly Leu Gln Ala Asn Thr Arg 180 185 190

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Met Thr Pro Val Met Lys Leu Gln Glu Asp Gln Lys Val Ser Asn Gly

Ala Arg Asn Arg Phe His Gly Leu Ala Asp Gln Arg Leu Val Ala Arg

Val Glu Asn Gly Asp Asp Cys Ser Ser Gly Ser Ser Val Thr Thr Ser

Asn Asn His Ser Val Asp Glu Ser Arg Ala Gln Ser Gly Ser Val Val

Glu Ala Gln Met Asn Asn Asn Asn Asn Asn Asn Met Asn Gly Tyr Ala

Cys Ile Pro Gly Val Pro Trp Pro Tyr Thr Trp Asn Pro Ala Met Pro

Pro Pro Gly Phe Tyr Pro Pro Pro Gly Tyr Pro Met Pro Phe Tyr Pro

Tyr Trp Thr Ile Pro Met Leu Pro Pro His Gln Ser Ser Pro Ile

Ser Gln Lys Cys Ser Asn Thr Asn Ser Pro Thr Leu Gly Lys His Pro

Arg Asp Glu Gly Ser Ser Lys Lys Asp Asn Glu Thr Glu Arg Lys Gln

Lys Ala Gly Cys Val Leu Val Pro Lys Thr Leu Arg Ile Asp Asp Pro

Asn Glu Ala Ala Lys Ser Ser Ile Trp Thr Thr Leu Gly Ile Lys Asn

Glu Ala Met Cys Lys Ala Gly Gly Met Phe Lys Gly Phe Asp His Lys

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			ctt Leu													240
			tct Ser													288
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			tct Ser													384
			cac His													432
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gcg gaa tca Ala Glu Ser	acg ggg c Thr Gly L 325	tg gac ca eu Asp Gl	n Lys G	ag ata In Ile	aac aat Asn Asn	tgg tto Trp Phe 335	Ile
aac cag agg Asn Gln Arg							
gta atg gac Val Met Asp 355	gca aca c Ala Thr H	at cct ca is Pro Hi 36	s His T	ac ttc Yr Phe	atg gat Met Asp 365	aat gto Asn Val	ttg 1104 Leu
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Ile Met Thr 35	Ser His G	ln His Hi 40	s Gly H	lis Asp	His Gln 45	His Gln	Gln
Gln Glu His 50	Asp Gly T	yr Ala Ty 55	r Gln S		His Gln 60	Gln Ser	Ser
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Val Ala Ser	Ser Ser S 85	er Pro Se		ys Ala 0	Pro Ala	Tyr Ser 95	Leu
Met Glu Ile	His His A	sn Glu Il	e Val A 105	la Gly	Gly Ile	Asn Pro	Cys
Ser Ser Phe 115	Ser Ser S	er Ala Se 12		ys Ala	Lys Ile 125	Met Ala	His
Pro His Tyr 130	His Arg L	eu Leu Ala 135	a Ala T	-	Asn Cys 140	Gln Lys	Val
Gly Ala Pro		al Val Ala 50	a Arg L	eu Glu 155	Glu Ala	Cys Ser	Ser 160
Ala Ala Ala	Ala Ala A 165	la Ser Me		ro Thr	Gly Cys	Leu Gly 175	Glu

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245 250 255

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576

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Ala Gln Gly Ile Arg Asp Arg Arg Val Arg Leu Phe Ile Gly Ile Ala 100 \$105\$

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Lys Thr Leu Asp Trp Leu Leu Lys Lys Ser Arg Lys Ala Ile Lys Glu 130 135 140

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Glu Pro Ile Glu Glu Phe Asp Asn Gln Glu Ser Ile Leu Thr Asn Met 275 280 285

Thr Leu Pro Thr Lys Met Gly Gln Ser Tyr Asn Gln Asn Asn Gly Ile 290 295 300

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		aat Asn														829
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Tyr Ala Thr Arg Arg Gly Cys Gly Val Cys Ile Ile Ser Gly Thr Gly

Ala Val Thr Asn Val Thr Ile Arg Gln Pro Ala Ala Pro Ala Gly Gly

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### MBI0018 Sequence Listing.ST25

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Met	Asn	Asn	Phe	Gln 245	Phe	Ser	Gly	Gly	Asp 250	Ile	Tyr	Gly	Met	Ser 255	Gly			
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ttt Phe																1	44	
ctc Leu																1	92	
aaa Lys 65																2	40	
cat His	gga Gly	ttc Phe	gat Asp	ata Ile 85	gta Val	atc Ile	agt Ser	gat Asp	gtt Val 90	cat His	atg Met	cct Pro	gac Asp	atg Met 95	gac Asp	2	88	
ggt Gly																3	36	
atc Ile																3	84	
acg Thr																4	32	
ctt Leu 145																4	80	
agt Ser																5	28	
cag	cag	caa	cat	aga	gga	ggt	ggt	ggt		gca age !	_	gtt	tct	ggt	gga	5	76	

Gln	Gln	Gln	His 180	Arg	Gly			018 Gly 185							Gly	
					gat Asp											624
tgg Trp	agg Arg 210	agc Ser	agt Ser	tca Ser	cgg Arg	aag Lys 215	agg Arg	aaa Lys	gac Asp	gag Glu	gaa Glu 220	gga Gly	gaa Glu	gag Glu	caa Gln	672
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					ctt Leu											1056
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					tca Ser 470											1440

	MBI0018 Sequenc						ongo	Tigting ST25								
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ttc ca Phe Gl	a cca n Pro 515	gaa Glu	ctt Leu	ccc Pro	gtg Val	aac Asn 520	agt Ser	ttc Phe	ccg Pro	ctg Leu	gca Ala 525	agt Ser	gca Ala	cca Pro		1584
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aac ag Asn Se 545	c tcc r Ser	gaa Glu	gcg Ala	ggt Gly 550	ttc Phe	att Ile	acg Thr	ccg Pro	agc Ser 555	tac Tyr	gac Asp	atg Met	ttc Phe	acc Thr 560		1680
acc ag Thr Ar	a cag g Gln	aat Asn	gat Asp 565	tgg Trp	gat Asp	ctg Leu	agg Arg	aat Asn 570	att Ile	gga Gly	ata Ile	gcc Ala	ttt Phe 575	gac Asp		1728
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tct tc Ser Se	t tcg r Ser 595	tcc Ser	atg Met	tca Ser	aga Arg	cac His 600	aac Asn	acg Thr	aca Thr	gtt Val	gca Ala 605	gcc Ala	acc Thr	gag Glu		1824
cat gg His Gl 61	y Arg	aac Asn	cac His	cag Gln	cag Gln 615	cca Pro	cca Pro	tcg Ser	gga Gly	atg Met 620	gta Val	cag Gln	cac His	cat His		1872
cag gt Gln Va 625	t tat l Tyr	gca Ala	gac Asp	gga Gly 630	aac Asn	ggt Gly	ggt Gly	tca Ser	gtg Val 635	agg Arg	gtg Val	aaa Lys	tca Ser	gag Glu 640		1920
aga gt Arg Va	g gct l Ala	acg Thr	gat Asp 645	aca Thr	gca Ala	aca Thr	atg Met	gcg Ala 650	ttt Phe	cac His	gag Glu	cag Gln	tat Tyr 655	agt Ser		1968
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Leu Me		Leu	Glu	Arg	Met 55	Leu	Arg	Thr	Cys	Leu 60	Tyr	Glu	Val	Thr		

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Lys Cys Asn Arg Ala Glu Met Ala Leu Ser Leu Leu Arg Lys Asn Lys 65 70 75 80

His Gly Phe Asp Ile Val Ile Ser Asp Val His Met Pro Asp Met Asp Gly Phe Lys Leu Glu His Val Gly Leu Glu Met Asp Leu Pro Val Ile Met Met Ser Ala Asp Asp Ser Lys Ser Val Val Leu Lys Gly Val Thr His Gly Ala Val Asp Tyr Leu Ile Lys Pro Val Arg Met Glu Ala

Leu Lys Asn Ile Trp Gln His Val Val Arg Lys Arg Arg Ser Glu Trp 145 150 155 160

Ser Val Pro Glu His Ser Gly Ser Ile Glu Glu Thr Gly Glu Arg Gln  $165 \hspace{1.5cm} 170 \hspace{1.5cm} 175$ 

Gln Gln Gln His Arg Gly Gly Gly Gly Gly Ala Ala Val Ser Gly Gly 180 185 190

Glu Asp Ala Val Asp Asp Asn Ser Ser Ser Val Asn Glu Gly Asn Asn 195 200 205

Trp Arg Ser Ser Ser Arg Lys Arg Lys Asp Glu Glu Gly Glu Glu Gln 210 215 220

Gly Asp Asp Lys Asp Glu Asp Ala Ser Asn Leu Lys Lys Pro Arg Val 225 230 235

Val Trp Ser Val Glu Leu His Gln Gln Phe Val Ala Ala Val As<br/>n Gln 245 250 255

Leu Gly Val Glu Lys Ala Val Pro Lys Lys Ile Leu Glu Leu Met Asn 260 265

Val Pro Gly Leu Thr Arg Glu Asn Val Ala Ser His Leu Gln Lys Tyr 275 280 285

Arg Ile Tyr Leu Arg Arg Leu Gly Gly Val Ser Gln His Gln Gly Asn 290 295 300

Leu Asn Asn Ser Phe Met Thr Gly Gln Asp Ala Ser Phe Gly Pro Leu 305 310 315

Ser Thr Leu Asn Gly Phe Asp Leu Gln Ala Leu Ala Val Thr Gly Gln 325 330 335

Leu Pro Ala Gln Ser Leu Ala Gln Leu Gln Ala Ala Gly Leu Gly Arg 340 345 350

Pro Ala Met Val Ser Lys Ser Gly Leu Pro Val Ser Ser Ile Val Asp 355 360 365

Glu Arg Ser Ile Phe Ser Phe Asp Asn Thr Lys Thr Arg Phe Gly Glu 370 375 380

### MBI0018 Sequence Listing.ST25

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Ser Val Gln Asn Asn Gly Met Leu Met Pro Leu Ala Gly Gln Gln Ser

Leu Pro Arg Gly Pro Pro Pro Met Leu Thr Ser Ser Gln Ser Ser Ile 450 455 460

Arg Gln Pro Met Leu Ser Asn Arg Ile Ser Glu Arg Ser Gly Phe Ser 465 470 475 480

Gly Arg Asn Asn Ile Pro Glu Ser Ser Arg Val Leu Pro Thr Ser Tyr 485 490 495

Thr Asn Leu Thr Thr Gln His Ser Ser Ser Ser Met Pro Tyr Asn Asn 500 505 510

Phe Gln Pro Glu Leu Pro Val Asn Ser Phe Pro Leu Ala Ser Ala Pro 515 520 525

Gly Ile Ser Val Pro Val Arg Lys Ala Thr Ser Tyr Gln Glu Glu Val 530 540

Asn Ser Ser Glu Ala Gly Phe Ile Thr Pro Ser Tyr Asp Met Phe Thr 545 555 5560

Thr Arg Gln Asn Asp Trp Asp Leu Arg Asn Ile Gly Ile Ala Phe Asp
565 570 575

Ser His Gln Asp Ser Glu Ser Ala Ala Phe Ser Ala Ser Glu Ala Tyr 580 585 590

Ser Ser Ser Met Ser Arg His Asn Thr Thr Val Ala Ala Thr Glu 595 600 605

His Gly Arg Asn His Gln Gln Pro Pro Ser Gly Met Val Gln His His 610 620

Gln Val Tyr Ala Asp Gly Asn Gly Gly Ser Val Arg Val Lys Ser Glu 625 635 640

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ttc gag aga tca acc gga cca gac cgg caa ttg tat atc cac tgg aaa Phe Glu Arg Ser Thr Gly Pro Asp Arg Gln Leu Tyr Ile His Trp Lys 290 295 300	914
gtc cgg tct agt ccg gtt cag act gtg gtt agg cta ttc gga gtc aac Val Arg Ser Ser Pro Val Gln Thr Val Val Arg Leu Phe Gly Val Asn 305 310 315	962
att ttc aat gtg agt aac gag aaa cca aac gac gtc gca gta gag tgt Ile Phe Asn Val Ser Asn Glu Lys Pro Asn Asp Val Ala Val Glu Cys 320 325 330 330	1010
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PCT/US00/31325 WO 01/36444

MBI0018 Sequence Listing.ST25 130

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Glu Gln Ser Arg Arg Lys Phe Val Asn Gly Asp Gly Lys Arg Ser Gly

Leu Glu Thr Ala Thr Tyr Gly Asn Asp Ala Val Leu Arg Ala Arg Glu

Val Leu Phe Glu Lys Thr Val Thr Pro Ser Asp Val Gly Lys Leu Asn

Arg Leu Val Ile Pro Lys Gln His Ala Glu Lys His Phe Pro Leu Pro

Ala Met Thr Thr Ala Met Gly Met Asn Pro Ser Pro Thr Lys Gly Val

Leu Ile Asn Leu Glu Asp Arg Thr Gly Lys Val Trp Arg Phe Arg Tyr

Ser Tyr Trp Asn Ser Ser Gln Ser Tyr Val Leu Thr Lys Gly Trp Ser

Arg Phe Val Lys Glu Lys Asn Leu Arg Ala Gly Asp Val Val Cys Phe

Glu Arg Ser Thr Gly Pro Asp Arg Gln Leu Tyr Ile His Trp Lys Val 290 295 300

Arg Ser Ser Pro Val Gln Thr Val Val Arg Leu Phe Gly Val Asn Ile

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60 108

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ggt Gly	gtg Val 65	gtg Val	cca Pro	caa Gln	cca Pro	aac Asn 70	gga Gly	aga Arg	tgg Trp	gga Gly	gct Ala 75	cag Gln	att Ile	tac Tyr	gag Glu	300	)
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									aaa Lys							540	)
									tcg Ser							588	3
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									aat Asn 265							876	5
									gat Asp							924	L
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Tyr Asn Glu Glu Leu Glu Gln Ser Lys Arg Arg Arg Asn Gly Asn Gly 145 150 155 160	
Asn Met Thr Arg Thr Leu Leu Thr Ser Gly Leu Ser Asn Asp Gly Val 165 170 175	
Ser Thr Thr Gly Phe Arg Ser Ala Glu Ala Leu Phe Glu Lys Ala Val 180 185 190	
Thr Pro Ser Asp Val Gly Lys Leu Asn Arg Leu Val Ile Pro Lys His 195 200 205	

## MBI0018 Sequence Listing.ST25

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Trp Ser Arg Phe 260	Val Lys G	u Lys Asn 265	Leu Arg A	Ala Gly Asp 270	Val Val
Ser Phe Ser Arg 275	Ser Asn G	y Gln Asp 280	Gln Gln L	Leu Tyr Ile 285	Gly Trp
Lys Ser Arg Ser 290	Gly Ser As			Arg Val Leu 300	Arg Leu
Phe Gly Val Asn 305	Ile Ser Pr 310	o Glu Ser	Ser Arg A	Asn Asp Val	Val Gly 320
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cta tat aga atg Leu Tyr Arg Met 30	gga agc gg Gly Ser Gl	y Thr Ser	Val Val L	ctt gat tca Leu Asp Ser 10	gag aac 207 Glu Asn
ggt gtc gaa gtc Gly Val Glu Val 45	gaa gtc ga Glu Val Gl 50	a gcc gaa u Ala Glu	tca aga a Ser Arg L 55	aag ctt cct Lys Leu Pro	tct tca 255 Ser Ser 60
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### MBI0018 Sequence Listing.ST25

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cgc Arg	gat Asp 110	gcc Ala	gtt Val	act Thr	aat Asn	ttc Phe 115	aaa Lys	gac Asp	acg Thr	acg Thr	ttc Phe 120	gaa Glu	gaa Glu	gag Glu	gtt Val	447
gag Glu 125	ttc Phe	tta Leu	aac Asn	gcg Ala	cat His 130	tcg Ser	aaa Lys	tca Ser	gag Glu	atc Ile 135	gta Val	gat Asp	atg Met	ttg Leu	aga Arg 140	495
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		gac Asp														687
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		tac Tyr														831
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atc Ile	agt Ser 270	ttt Phe	aaa Lys	aga Arg	tcc Ser	aac Asn 275	gat Asp	caa Gln	gat Asp	caa Gln	aaa Lys 280	ttc Phe	ttt Phe	atc Ile	ggg Gly	927
		tcg Ser														975
ttg Leu	ttt Phe	gly ggg	gtt Val	gat Asp 305	att Ile	tct Ser	tta Leu	aac Asn	gcc Ala 310	gtc Val	gtt Val	gta Val	gtg Val	aag Lys 315	gaa Glu	1023
aca Thr	acg Thr	gag Glu	gtg Val 320	tta Leu	atg Met	tcg Ser	tcg Ser	tta Leu 325	agg Arg	tgt Cys	aag Lys	aag Lys	caa Gln 330	cga Arg	gtt Val	1071
ttg Leu	taa	taad	caatt	ta a	acaad	ettg	gg aa	aagaa	aaaa	a aag	gcttt	ttg	atti	taat	:tt	1127
ctc	ttcaa	acg t	taat	ctt	gc to	gagat	ta									1155

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<400> 38

MBI0018 Sequence Listing.ST25 Met Asp Ala Met Ser Ser Val Asp Glu Ser Ser Thr Thr Thr Asp Ser Ile Pro Ala Arg Lys Ser Ser Ser Pro Ala Ser Leu Leu Tyr Arg Met 20 25 30Gly Ser Gly Thr Ser Val Val Leu Asp Ser Glu Asn Gly Val Glu Val Glu Val Glu Ala Glu Ser Arg Lys Leu Pro Ser Ser Arg Phe Lys Gly Val Val Pro Gln Pro Asn Gly Arg Trp Gly Ala Gln Ile Tyr Glu Lys His Gln Arg Val Trp Leu Gly Thr Phe Asn Glu Glu Asp Glu Ala Ala Arg Ala Tyr Asp Val Ala Ala His Arg Phe Arg Gly Arg Asp Ala Val Thr Asn Phe Lys Asp Thr Thr Phe Glu Glu Glu Val Glu Phe Leu Asn Ala His Ser Lys Ser Glu Ile Val Asp Met Leu Arg Lys His Thr Tyr Lys Glu Glu Leu Asp Gln Arg Lys Arg Asn Arg Asp Gly Asn Gly Lys 145 150 155 160Glu Thr Thr Ala Phe Ala Leu Ala Ser Met Val Val Met Thr Gly Phe Lys Thr Ala Glu Leu Leu Phe Glu Lys Thr Val Thr Pro Ser Asp Val Gly Lys Leu Asn Arg Leu Val Ile Pro Lys His Gln Ala Glu Lys His Phe Pro Leu Pro Leu Gly Asn Asn Asn Val Ser Val Lys Gly Met Leu 210 215 220 Leu Asn Phe Glu Asp Val Asn Gly Lys Val Trp Arg Phe Arg Tyr Ser Tyr Trp Asn Ser Ser Gln Ser Tyr Val Leu Thr Lys Gly Trp Ser Arg Phe Val Lys Glu Lys Arg Leu Cys Ala Gly Asp Leu Ile Ser Phe Lys Arg Ser Asn Asp Gln Asp Gln Lys Phe Phe Ile Gly Trp Lys Ser Lys Ser Gly Leu Asp Leu Glu Thr Gly Arg Val Met Arg Leu Phe Gly Val 290 295 300

### MBI0018 Sequence Listing.ST25

Asp	Ile	Ser	Leu	Asn	Ala	Val	Val	Val	Val	Lys	Glu	Thr	Thr	Glu	Val
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	)(10 .)(10	35)													
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atg atg t Met Met S															96
cta tgt t Leu Cys S 3	cc tcc er Ser 5	gcc (	ggt Gly	gaa Glu	aat Asn 40	cgt Arg	gtc Val	tct Ser	gat Asp	gtt Val 45	ttc Phe	gga Gly	tcc Ser	1	44
gac gag c Asp Glu L 50	ta ctc eu Leu	tca ( Ser	gta Val	gcc Ala 55	gtc Val	tcc Ser	gct Ala	ttg Leu	tcg Ser 60	tcg Ser	gag Glu	gcg Ala	gct Ala	1	92
tcg atc g Ser Ile A 65		Glu												. 2	40
gtc atc a Val Ile L														2	88
caa gct t Gln Ala T														3	36
tgt tta c Cys Leu L 1	ta gag eu Glu 15	gag Glu	att Ile	caa Gln	cgg Arg 120	gag Glu	agt Ser	gat Asp	gtt Val	tat Tyr 125	aag Lys	caa Gln	gag Glu	3	84
gtt gtt c Val Val P 130														4	32
atg gaa a Met Glu T 145		Cys												4	80
aga ccg t Arg Pro P														5	28
cta cgg a Leu Arg A	ac cta sn Leu 180	tgt ( Cys '	act Thr	ggt Gly	gtc Val	gag Glu 185	tct Ser	gcc Ala	agg Arg	gga Gly	gtt Val 190	tct Ser	ggg ggg	5	76
ggg atg t Gly Met S 1	ct cct er Pro 95	cat (	G1 y 999	gac Asp	aag Lys 200	act Thr	att Ile	agt Ser	cct Pro	ctc Leu 205	ctg Leu	aca Thr	aat Asn	6	24
gac aat g Asp Asn G	ga gag ly Glu	gat (	ggt Gly	gta Val	ata Ile	tca Ser	Ser	gac Asp age	Glu	gaa Glu	ctg Leu	agt Ser	gga Gly	6	72

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gac ctc aaa gat agg ttg Asp Leu Lys Asp Arg Leu 245	Leu Arg Lys I	ttt gga agc cgt Phe Gly Ser Arg 250	att agt act Ile Ser Thr 255	768
tta aag ctt gag ttc tca Leu Lys Leu Glu Phe Ser 260				816
gaa gca aga caa gct ctt Glu Ala Arg Gln Ala Leu 275				864
cct tac cct act gaa gga Pro Tyr Pro Thr Glu Gly 290	gat aag ata g Asp Lys Ile A 295	gca tta gct gat Ala Leu Ala Asp 300	gca acg ggg Ala Thr Gly	912
tta gac caa aaa caa atc Leu Asp Gln Lys Gln Ile 305 310				960
cat tgg aag cca tca gag His Trp Lys Pro Ser Glu 325	Asn Met Pro P			1008
agt gga tca ttc ttt acc Ser Gly Ser Phe Phe Thr 340			=	1035
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	iana			
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<pre>&lt;213&gt; Arabidopsis thal &lt;400&gt; 40  Met Tyr Asn Phe His Ser 1</pre>	Ala Gly Asp Tangle Leu Met Phe F 25 Glu Asn Arg V 40 Ala Val Ser A	Pro Ser Asp Tyr Val Ser Asp Val 45 Ala Leu Ser Ser	Gln Ala Leu 30 Phe Gly Ser Glu Ala Ala	
<pre>&lt;213&gt; Arabidopsis thal &lt;400&gt; 40  Met Tyr Asn Phe His Ser 1</pre>	Ala Gly Asp The English Asp Arg Arg Arg Asn Arg Asn And And Arg And And And And And And And And And And	Pro Ser Asp Tyr  Val Ser Asp Val 45  Ala Leu Ser Ser 60  Asp Asp Asn Val 75	Gln Ala Leu 30  Phe Gly Ser  Glu Ala Ala  Ser Leu Thr 80	
<pre>&lt;213&gt; Arabidopsis thal &lt;400&gt; 40  Met Tyr Asn Phe His Ser 1</pre>	Ala Gly Asp Tall Leu Met Phe F 25 Glu Asn Arg V 40 Ala Val Ser A 55 Arg Arg Asn A Ala Cys His F	Pro Ser Asp Tyr  Val Ser Asp Val 45  Ala Leu Ser Ser 60  Asp Asp Asn Val 75	Gln Ala Leu 30  Phe Gly Ser  Glu Ala Ala  Ser Leu Thr 80  Arg Leu Leu 95	
<pre>&lt;213&gt; Arabidopsis thal &lt;400&gt; 40  Met Tyr Asn Phe His Ser 1</pre>	Ala Gly Asp The Leu Met Phe Formula 25 Glu Asn Arg Valor Asp Arg Arg Arg Asn Arg Arg Asn Arg Ala Cys His Formula 25 Gln Lys Valor Arg 105	Pro Ser Asp Tyr  Val Ser Asp Val 45  Ala Leu Ser Ser 60  Asp Asp Asn Val 75  Pro Ser Tyr Pro 90  Gly Ala Pro Pro	Gln Ala Leu 30  Phe Gly Ser  Glu Ala Ala  Ser Leu Thr 80  Arg Leu Leu 95  Glu Ile Ala 110	

MBI0018 Sequence Listing.ST25

Met Glu Thr Tyr Cys Asp Ile Leu Val Lys Tyr Lys Ser Asp Leu Ala

Arg Pro Phe Asp Glu Ala Thr Cys Phe Leu Asn Lys Ile Glu Met Gln

Leu Arg Asn Leu Cys Thr Gly Val Glu Ser Ala Arg Gly Val Ser Gly

Gly Met Ser Pro His Gly Asp Lys Thr Ile Ser Pro Leu Leu Thr Asn

Asp Asn Gly Glu Asp Gly Val Ile Ser Ser Asp Glu Glu Leu Ser Gly

Gly Asp His Glu Val Ala Glu Asp Gly Arg Gln Arg Cys Glu Asp Arg

Asp Leu Lys Asp Arg Leu Leu Arg Lys Phe Gly Ser Arg Ile Ser Thr

Leu Lys Leu Glu Phe Ser Lys Lys Lys Lys Gly Lys Leu Pro Arg

Glu Ala Arg Gln Ala Leu Leu Asp Trp Trp Asn Leu His Tyr Lys Trp

Pro Tyr Pro Thr Glu Gly Asp Lys Ile Ala Leu Ala Asp Ala Thr Gly

Leu Asp Gln Lys Gln Ile Asn Asn Trp Phe Ile Asn Gln Arg Lys Arg

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Ser Gly Ser Phe Phe Thr Glu Glu 340

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ccg gat aaa ggg tta gat tcc ggc aag tat gtg agg tac acg ccg gag Pro Asp Lys Gly Leu Asp Ser Gly Lys Tyr Val Arg Tyr Thr Pro Glu 20 25 30

96

caa Gln	gtg Val	gaa Glu 35	gct Ala	ctc Leu	gag Glu	aga	qtt	tac	Sequ act Thr	gag	tgt	cct	aag	cca	agt Ser	144
tct Ser	cta Leu 50	aga Arg	aga Arg	caa Gln	caa Gln	ctc Leu 55	ata Ile	cgt Arg	gaa Glu	tgt Cys	ccg Pro 60	att Ile	ctc Leu	tct Ser	aac Asn	192
atc Ile 65	gag Glu	cct Pro	aag Lys	cag Gln	atc Ile 70	aaa Lys	gtt Val	tgg Trp	ttt Phe	cag Gln 75	aac Asn	cgc Arg	aga Arg	tgt Cys	cgt Arg 80	240
gag Glu	aag Lys	cag Gln	agg Arg	aaa Lys 85	gaa Glu	gct Ala	gct Ala	cgt Arg	ctt Leu 90	caa Gln	aca Thr	gtg Val	aac Asn	aga Arg 95	aaa Lys	288
ctc Leu	aat Asn	gcc Ala	atg Met 100	aac Asn	aaa Lys	ctc Leu	ttg Leu	atg Met 105	gaa Glu	gag Glu	aat Asn	gat Asp	cgt Arg 110	ttg Leu	cag Gln	336
aag Lys	caa Gln	gtt Val 115	tct Ser	aac Asn	ttg Leu	gtc Val	tat Tyr 120	gag Glu	aat Asn	ggc Gly	cac His	atg Met 125	aaa Lys	cat His	caa Gln	384
									gac Asp							432
									caa Gln							480
									gga Gly 170							528
gag Glu	gcc Ala	cta Leu	gca Ala 180	gag Glu	ttc Phe	ctt Leu	tcc Ser	aag Lys 185	gct Ala	aca Thr	gga Gly	act Thr	gct Ala 190	gtt Val	gac Asp	576
tgg Trp	gtt Val	cag Gln 195	atg Met	att Ile	Gly 999	atg Met	aag Lys 200	cct Pro	ggt Gly	ccg Pro	gat Asp	tct Ser 205	att Ile	ggc Gly	ata Ile	624
gtc Val	gct Ala 210	att Ile	tcg Ser	cgc Arg	aac Asn	tgc Cys 215	agc Ser	gga Gly	att Ile	gca Ala	gca Ala 220	cgt Arg	gcc Ala	tgc Cys	ggc Gly	672
									gct Ala							720
cca Pro	tct Ser	tgg Trp	ctc Leu	cga Arg 245	gat Asp	tgt Cys	cga Arg	agt Ser	gtg Val 250	gat Asp	act Thr	ctg Leu	agt Ser	gtg Val 255	ata Ile	768
cct Pro	gct Ala	gga Gly	aac Asn 260	ggt Gly	gly ggg	acg Thr	atc Ile	gag Glu 265	ctt Leu	att Ile	tac Tyr	acg Thr	cag Gln 270	atg Met	tat Tyr	816
									gac Asp							864
agc Ser	aca Thr 290	tgt Cys	ttg Leu	gaa Glu	gat Asp	gga Gly 295	agc Ser	tat Tyr	gtg Val	gtt Val	tgt Cys 300	gaa Glu	agg Arg	tcg Ser	ctt Leu	912
									cca Pro							960
aga Arg	gct Ala	gaa Glu	atg Met	aaa Lys 325	cca Pro	agc Ser	G1A aaa	ttt Phe	ctc Leu 330	Ile	cgt Arg	cct Pro	tgc Cys	gat Asp 335	ggt Gly	1008

## MBI0018 Sequence Listing.ST25

ggt Gly	ggt Gly	tcc Ser	att Ile 340	ctc Leu	cac His	att Ile	gtt Val	gat Asp 345	cat His	gtt Val	gat Asp	ctg Leu	gat Asp 350	gcc Ala	tgg Trp	1056
agt Ser	gtc Val	cct Pro 355	gaa Glu	gtc Val	atg Met	agg Arg	cct Pro 360	ctc Leu	tat Tyr	gaa Glu	tca Ser	tcg Ser 365	aag Lys	att Ile	ctt Leu	1104
gct Ala	cag Gln 370	aaa Lys	atg Met	act Thr	gtt Val	gct Ala 375	gct Ala	ttg Leu	aga Arg	cat His	gta Val 380	aga Arg	caa Gln	att Ile	gca Ala	1152
caa Gln 385	gaa Glu	aca Thr	agt Ser	gga Gly	gaa Glu 390	gtt Val	cag Gln	tat Tyr	ggt Gly	gga Gly 395	Gly ggg	cgc Arg	caa Gln	cct Pro	gcg Ala 400	1200
gtt Val	tta Leu	aga Arg	acc Thr	ttc Phe 405	agt Ser	caa Gln	aga Arg	ctc Leu	tgt Cys 410	cgg Arg	ggt Gly	ttc Phe	aat Asn	gat Asp 415	gct Ala	1248
gtt Val	aat Asn	ggt Gly	ttt Phe 420	gtg Val	gat Asp	gat Asp	gga Gly	tgg Trp 425	tca Ser	cca Pro	atg Met	ggt Gly	agc Ser 430	gat Asp	ggt Gly	1296
gca Ala	gag Glu	gat Asp 435	gtt Val	act Thr	gta Val	atg Met	ata Ile 440	aac Asn	ttg Leu	tcc Ser	cct Pro	999 Gly 445	aag Lys	ttt Phe	ggt Gly	1344
G1y 999	tct Ser 450	cag Gln	tac Tyr	ggt Gly	aat Asn	tca Ser 455	ttc Phe	ctt Leu	cca Pro	agc Ser	ttt Phe 460	ggt Gly	agt Ser	ggc Gly	gtg Val	1392
ctt Leu 465	tgt Cys	gcc Ala	aag Lys	gca Ala	tct Ser 470	atg Met	ttg Leu	ctt Leu	cag Gln	aac Asn 475	gtt Val	cca Pro	ccc Pro	gct Ala	gtg Val 480	1440
ctg Leu	gtt Val	cga Arg	ttc Phe	ctt Leu 485	aga Arg	gaa Glu	cac His	cga Arg	tct Ser 490	gaa Glu	tgg Trp	gct Ala	gat Asp	tat Tyr 495	ggc Gly	1488
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ctt Leu	gcg Ala 530	cag Gln	aca Thr	gtt Val	gaa Glu	cat His 535	gaa Glu	gag Glu	tca Ser	ctt Leu	gag Glu 540	gtg Val	gtt Val	aga Arg	ctt Leu	1632
gaa Glu 545	ggt Gly	cac His	gct Ala	tac Tyr	tca Ser 550	ccc Pro	gaa Glu	gac Asp	atg Met	ggt Gly 555	tta Leu	gct Ala	cgg Arg	gat Asp	atg Met 560	1680
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tgt Cys	gca Ala	cag Gln	ctt Leu 580	gta Val	ttt Phe	gcc Ala	cct Pro	atc Ile 585	gat Asp	gaa Glu	tca Ser	ttt Phe	gct Ala 590	gat Asp	gat Asp	1776
gca Ala	cct Pro	ttg Leu 595	ctt Leu	cct Pro	tcc Ser	ggt Gly	ttc Phe 600	cgc Arg	atc Ile	ata Ile	cct Pro	ctt Leu 605	gaa Glu	cag Gln	aaa Lys	1824
tct Ser	act Thr 610	ccg Pro	aac Asn	ggt Gly	gca Ala	tct Ser 615	gca Ala	aac Asn	cgt Arg	acc Thr	ctg Leu 620	gat Asp	tta Leu	gcc Ala	tca Ser	1872
gct Ala	tta Leu	gaa Glu	gga Gly	tcc Ser	aca Thr	cgt Arg	caa Gln	gct Ala	Gly	gaa Glu age	Ala	gac Asp	cca Pro	aat Asn	ggc Gly	1920

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aac cat tca aga gac agt gtt gct tca atg gca cgt cag tac gtg cga Asn His Ser Arg Asp Ser Val Ala Ser Met Ala Arg Gln Tyr Val Arg 660 665 670	2016
agc ata gta gga tcg att cag agg gtt gct cta gcc att gct cct cgt Ser Ile Val Gly Ser Ile Gln Arg Val Ala Leu Ala Ile Ala Pro Arg 675 680 685	2064
cct ggc tcc aat atc agt cca ata tct gtt ccc act tcc cct gaa gct Pro Gly Ser Asn Ile Ser Pro Ile Ser Val Pro Thr Ser Pro Glu Ala 690 695 700	2112
ctc act ctg gtc cgt tgg atc tcc cgg agt tac agc ctt cac act ggt Leu Thr Leu Val Arg Trp Ile Ser Arg Ser Tyr Ser Leu His Thr Gly 705 710 715 720	2160
gca gat ctc ttt gga tct gat tct caa acc agt ggt gac acg ttg ctg Ala Asp Leu Phe Gly Ser Asp Ser Gln Thr Ser Gly Asp Thr Leu Leu 725 730 735	2208
cat caa ctc tgg aat cac tct gat gca atc ttg tgc tgc tcc ctc aaa His Gln Leu Trp Asn His Ser Asp Ala Ile Leu Cys Cys Ser Leu Lys 740 745 750	2256
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Pro Asp Lys Gly Leu Asp Ser Gly Lys Tyr Val Arg Tyr Thr Pro Glu 20 25 30	
Gln Val Glu Ala Leu Glu Arg Val Tyr Thr Glu Cys Pro Lys Pro Ser 35 40 45	
Ser Leu Arg Arg Gln Gln Leu Ile Arg Glu Cys Pro Ile Leu Ser Asn 50 55 60	
Ile Glu Pro Lys Gln Ile Lys Val Trp Phe Gln Asn Arg Arg Cys Arg 65 70 75 80	

#### MBI0018 Sequence Listing.ST25

Leu Asn Ala Met Asn Lys Leu Leu Met Glu Glu Asn Asp Arg Leu Gln Lys Gln Val Ser Asn Leu Val Tyr Glu Asn Gly His Met Lys His Gln Leu His Thr Ala Ser Gly Thr Thr Thr Asp Asn Ser Cys Glu Ser Val Val Val Ser Gly Gln Gln His Gln Gln Gln Asn Pro Asn Pro Gln His Gln Gln Arg Asp Ala Asn Asn Pro Ala Gly Leu Leu Ser Ile Ala Glu Glu Ala Leu Ala Glu Phe Leu Ser Lys Ala Thr Gly Thr Ala Val Asp Trp Val Gln Met Ile Gly Met Lys Pro Gly Pro Asp Ser Ile Gly Ile Val Ala Ile Ser Arg Asn Cys Ser Gly Ile Ala Ala Arg Ala Cys Gly 210 215 220 Leu Val Ser Leu Glu Pro Met Lys Val Ala Glu Ile Leu Lys Asp Arg Pro Ser Trp Leu Arg Asp Cys Arg Ser Val Asp Thr Leu Ser Val Ile Pro Ala Gly Asn Gly Gly Thr Ile Glu Leu Ile Tyr Thr Gln Met Tyr Ala Pro Thr Thr Leu Ala Ala Ala Arg Asp Phe Trp Thr Leu Arg Tyr Ser Thr Cys Leu Glu Asp Gly Ser Tyr Val Val Cys Glu Arg Ser Leu 290 295 300 Thr Ser Ala Thr Gly Gly Pro Thr Gly Pro Pro Ser Ser Asn Phe Val Arg Ala Glu Met Lys Pro Ser Gly Phe Leu Ile Arg Pro Cys Asp Gly Gly Gly Ser Ile Leu His Ile Val Asp His Val Asp Leu Asp Ala Trp Ser Val Pro Glu Val Met Arg Pro Leu Tyr Glu Ser Ser Lys Ile Leu Ala Gln Lys Met Thr Val Ala Ala Leu Arg His Val Arg Gln Ile Ala Gln Glu Thr Ser Gly Glu Val Gln Tyr Gly Gly Gly Arg Gln Pro Ala 395

#### MBI0018 Sequence Listing.ST25

Val Leu Arg Thr Phe Ser Gln Arg Leu Cys Arg Gly Phe Asn Asp Ala Val Asn Gly Phe Val Asp Asp Gly Trp Ser Pro Met Gly Ser Asp Gly Ala Glu Asp Val Thr Val Met Ile Asn Leu Ser Pro Gly Lys Phe Gly Gly Ser Gln Tyr Gly Asn Ser Phe Leu Pro Ser Phe Gly Ser Gly Val Leu Cys Ala Lys Ala Ser Met Leu Leu Gln Asn Val Pro Pro Ala Val Leu Val Arg Phe Leu Arg Glu His Arg Ser Glu Trp Ala Asp Tyr Gly Val Asp Ala Tyr Ala Ala Ala Ser Leu Arg Ala Ser Pro Phe Ala Val Pro Cys Ala Arg Ala Gly Gly Phe Pro Ser Asn Gln Val Ile Leu Pro Leu Ala Gln Thr Val Glu His Glu Glu Ser Leu Glu Val Val Arg Leu Glu Gly His Ala Tyr Ser Pro Glu Asp Met Gly Leu Ala Arg Asp Met Tyr Leu Leu Gln Leu Cys Ser Gly Val Asp Glu Asn Val Val Gly Gly Cys Ala Gln Leu Val Phe Ala Pro Ile Asp Glu Ser Phe Ala Asp Asp Ala Pro Leu Leu Pro Ser Gly Phe Arg Ile Ile Pro Leu Glu Gln Lys Ser Thr Pro Asn Gly Ala Ser Ala Asn Arg Thr Leu Asp Leu Ala Ser Ala Leu Glu Gly Ser Thr Arg Gln Ala Gly Glu Ala Asp Pro Asn Gly Cys Asn Phe Arg Ser Val Leu Thr Ile Ala Phe Gln Phe Thr Phe Asp Asn His Ser Arg Asp Ser Val Ala Ser Met Ala Arg Gln Tyr Val Arg Ser Ile Val Gly Ser Ile Gln Arg Val Ala Leu Ala Ile Ala Pro Arg Pro Gly Ser Asn Ile Ser Pro Ile Ser Val Pro Thr Ser Pro Glu Ala Page 71

MBI0018 Sequence Listing.ST25 690 695 700

Leu Thr Leu Val Arg Trp Ile Ser Arg Ser Tyr Ser Leu His Thr Gly Ala Asp Leu Phe Gly Ser Asp Ser Gln Thr Ser Gly Asp Thr Leu Leu His Gln Leu Trp Asn His Ser Asp Ala Ile Leu Cys Cys Ser Leu Lys Thr <210> 43 <211> 2526 <212> DNA 2526 <213> Arabidopsis thaliana <220> <221> CDS <222> (1)..(2526) <223> G390 atg atg gct cat cac tcc atg gac gat aga gac tct cct gat aaa gga Met Met Ala His His Ser Met Asp Asp Asp Ser Pro Asp Lys Gly 48 ttt gat tcc ggc aag tac gtt aga tac acg ccg gaa caa gtt gaa gct Phe Asp Ser Gly Lys Tyr Val Arg Tyr Thr Pro Glu Gln Val Glu Ala 96 ctt gag aga gtt tat gct gag tgt cct aaa cct agc tct ctg aga aga Leu Glu Arg Val Tyr Ala Glu Cys Pro Lys Pro Ser Ser Leu Arg Arg 144 caa cag ctt att cgt gaa tgt ccc att ctc tgt aac atc gag cct cga Gln Gln Leu Ile Arg Glu Cys Pro Ile Leu Cys Asn Ile Glu Pro Arg 192 cag atc aaa gtt tgg ttc cag aat cgc aga tgt cga gag aag cag agg Gln Ile Lys Val Trp Phe Gln Asn Arg Arg Cys Arg Glu Lys Gln Arg 65 70 75 80 240 aaa gag tca gct cgt ctt cag aca gtg aac agg aag ctg agt gct atg Lys Glu Ser Ala Arg Leu Gln Thr Val Asn Arg Lys Leu Ser Ala Met 288 aac aag ctt ttg atg gaa gag aat gat cgt ttg cag aag caa gtc tcc Asn Lys Leu Leu Met Glu Glu Asn Asp Arg Leu Gln Lys Gln Val Ser 336 aac ttg gtt tat gag aat gga ttc atg aaa cat cga atc cac act gct Asn Leu Val Tyr Glu Asn Gly Phe Met Lys His Arg Ile His Thr Ala 384 tct ggg acg acc aca gac aac agc tgt gag tct gtg gtc gtg agt ggt Ser Gly Thr Thr Thr Asp Asp Ser Cys Glu Ser Val Val Val Ser Gly 432 135 cag caa cgt cag cag caa aac cca aca cat cag cat cct cag cgt gat 480 Gln Gln Arg Gln Gln Gln Asn Pro Thr His Gln His Pro Gln Arg Asp 155 150

gtt aac aac cca gct aat ctt ctc tcg att gcg gag gag acc ttg gcg Val Asn Asn Pro Ala Asn Leu Leu Ser Ile Ala Glu Glu Thr Leu Ala 528

							MBT0	018	Seau	ence	Lis	ting	.ST2	5		
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att Ile	999 Gly	atg Met 195	aag Lys	cct Pro	ggt Gly	ccg Pro	gat Asp 200	tct Ser	att Ile	ggt Gly	atc Ile	gta Val 205	gct Ala	gtt Val	tca Ser	624
cgc Arg	aac Asn 210	tgc Cys	agt Ser	gga Gly	ata Ile	gca Ala 215	gca Ala	cgt Arg	gcc Ala	tgt Cys	ggc Gly 220	ctc Leu	gtg Val	agt Ser	tta Leu	672
					gct Ala 230											720
cgt Arg	gac Asp	tgt Cys	cga Arg	tgt Cys 245	gtc Val	gag Glu	act Thr	ctg Leu	aat Asn 250	gtt Val	ata Ile	ccc Pro	act Thr	gga Gly 255	aat Asn	768
ggt Gly	ggt Gly	act Thr	atc Ile 260	gag Glu	ctt Leu	gtc Val	aac Asn	act Thr 265	cag Gln	att Ile	tat Tyr	gct Ala	cct Pro 270	aca Thr	aca Thr	816
					gac Asp											864
gaa Glu	gat Asp 290	gga Gly	agc Ser	tat Tyr	gtg Val	gtc Val 295	tgt Cys	gag Glu	aga Arg	tca Ser	ctc Leu 300	act Thr	tct Ser	gca Ala	act Thr	912
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ctg Leu	tca Ser	agc Ser	Gly ggg	ttt Phe 325	ctt Leu	atc Ile	cgt Arg	cct Pro	tgt Cys 330	gat Asp	ggt Gly	ggt Gly	ggt Gly	tcc Ser 335	att Ile	1008
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act Thr	gtc Val 370	gct Ala	gct Ala	ctg Leu	aga Arg	cat His 375	gtg Val	cgc Arg	caa Gln	att Ile	gct Ala 380	caa Gln	gag Glu	act Thr	agt Ser	1152
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acg Thr	atc Ile	atg Met 435	att Ile	aac Asn	tct Ser	tcc Ser	tct Ser 440	gct Ala	aaa Lys	ttt Phe	gct Ala	ggc Gly 445	tcc Ser	caa Gln	tac Tyr	1344
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					cag Gln 470											1440

### MBI0018 Sequence Listing.ST25

									-			_				
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											gtt Val					1536
acc Thr	ggt Gly	999 Gly 515	ttc Phe	ccg Pro	agt Ser	aac Asn	caa Gln 520	gtc Val	att Ile	ctt Leu	cct Pro	ctc Leu 525	gca Ala	cag Gln	aca Thr	1584
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ctt Leu	tgt Cys	agc Ser	ggc Gly	gtt Val 565	gat Asp	gaa Glu	aat Asn	gtg Val	gtt Val 570	gga Gly	ggt Gly	tgt Cys	gct Ala	cag Gln 575	ctt Leu	1728
gtc Val	ttt Phe	gcc Ala	cca Pro 580	atc Ile	gat Asp	gaa Glu	tca Ser	ttt Phe 585	gct Ala	gat Asp	gat Asp	gca Ala	cct Pro 590	ttg Leu	ctt Leu	1776
											aaa Lys					1824
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ggt Gly 625	tcc Ser	acc Thr	aaa Lys	acc Thr	gat Asp 630	tcg Ser	gaa Glu	aca Thr	aac Asn	tct Ser 635	aga Arg	ttg Leu	gtc Val	tta Leu	aca Thr 640	1920
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											ggt Gly					2016
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											acc Thr 700					2112
											tct Ser					2160
gga Gly	gac Asp	aca Thr	ttg Leu	ctt Leu 725	aag Lys	caa Gln	ctc Leu	tgg Trp	gac Asp 730	cat His	agt Ser	gat Asp	gcc Ala	ata Ile 735	ttg Leu	2208
tgc Cys	tgc Cys	tcc Ser	ctg Leu 740	aaa Lys	act Thr	aat Asn	gcc Ala	tca Ser 745	ccg Pro	gta Val	ttc Phe	aca Thr	ttt Phe 750	gca Ala	aac Asn	2256
											gtg Val					2304
ata Ile	atg Met	ctc Leu	gac Asp	aaa Lys	aca Thr	ctt Leu	gat Asp	gac Asp	Ser	ggt Gly	cgt Arg	aga Arg	gct Ala	ctt Leu	tgc Cys	2352

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Gln Ile Lys Val Trp Phe Gln Asn Arg Arg Cys Arg Glu Lys Gln Arg 65 70 75 80	
Lys Glu Ser Ala Arg Leu Gln Thr Val Asn Arg Lys Leu Ser Ala Met 85 90 95	
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Ser Gly Thr Thr Thr Asp Asn Ser Cys Glu Ser Val Val Ser Gly 130 135 140	
Gln Gln Arg Gln Gln Gln Asn Pro Thr His Gln His Pro Gln Arg Asp 145 150 155 160	
Val Asn Asn Pro Ala Asn Leu Leu Ser Ile Ala Glu Glu Thr Leu Ala 165 170 175	
Glu Phe Leu Cys Lys Ala Thr Gly Thr Ala Val Asp Trp Val Gln Met 180 185 190	
Ile Gly Met Lys Pro Gly Pro Asp Ser Ile Gly Ile Val Ala Val Ser	

# MBI0018 Sequence Listing.ST25

195

Arg Asn Cys Ser Gly Ile Ala Ala Arg Ala Cys Gly Leu Val Ser Leu Glu Pro Met Lys Val Ala Glu Ile Leu Lys Asp Arg Pro Ser Trp Phe Arg Asp Cys Arg Cys Val Glu Thr Leu Asn Val Ile Pro Thr Gly Asn Gly Gly Thr Ile Glu Leu Val Asn Thr Gln Ile Tyr Ala Pro Thr Thr Leu Ala Ala Arg Asp Phe Trp Thr Leu Arg Tyr Ser Thr Ser Leu Glu Asp Gly Ser Tyr Val Val Cys Glu Arg Ser Leu Thr Ser Ala Thr Gly Gly Pro Asn Gly Pro Leu Ser Ser Ser Phe Val Arg Ala Lys Met Leu Ser Ser Gly Phe Leu Ile Arg Pro Cys Asp Gly Gly Ser Ile Ile His Ile Val Asp His Val Asp Leu Asp Val Ser Ser Val Pro Glu Val Leu Arg Pro Leu Tyr Glu Ser Ser Lys Ile Leu Ala Gln Lys Met Thr Val Ala Ala Leu Arg His Val Arg Gln Ile Ala Gln Glu Thr Ser Gly Glu Val Gln Tyr Ser Gly Gly Arg Gln Pro Ala Val Leu Arg Thr Phe Ser Gln Arg Leu Cys Arg Gly Phe Asn Asp Ala Val Asn Gly Phe Val Asp Asp Gly Trp Ser Pro Met Ser Ser Asp Gly Gly Glu Asp Ile Thr Ile Met Ile Asn Ser Ser Ser Ala Lys Phe Ala Gly Ser Gln Tyr Gly Ser Ser Phe Leu Pro Ser Phe Gly Ser Gly Val Leu Cys Ala Lys Ala Ser Met Leu Leu Gln Asn Val Pro Pro Leu Val Leu Ile Arg Phe Leu Arg Glu His Arg Ala Glu Trp Ala Asp Tyr Gly Val Asp Ala Tyr 490

MBI0018 Sequence Listing.ST25 Ser Ala Ala Ser Leu Arg Ala Thr Pro Tyr Ala Val Pro Cys Val Arg Thr Gly Gly Phe Pro Ser Asn Gln Val Ile Leu Pro Leu Ala Gln Thr Leu Glu His Glu Glu Phe Leu Glu Val Val Arg Leu Gly Gly His Ala Tyr Ser Pro Glu Asp Met Gly Leu Ser Arg Asp Met Tyr Leu Leu Gln Leu Cys Ser Gly Val Asp Glu Asn Val Val Gly Gly Cys Ala Gln Leu Val Phe Ala Pro Ile Asp Glu Ser Phe Ala Asp Asp Ala Pro Leu Leu Pro Ser Gly Phe Arg Val Ile Pro Leu Asp Gln Lys Thr Asn Pro Asn 600 Asp His Gln Ser Ala Ser Arg Thr Arg Asp Leu Ala Ser Ser Leu Asp Gly Ser Thr Lys Thr Asp Ser Glu Thr Asn Ser Arg Leu Val Leu Thr Ile Ala Phe Gln Phe Thr Phe Asp Asn His Ser Arg Asp Asn Val Ala Thr Met Ala Arg Gln Tyr Val Arg Asn Val Val Gly Ser Ile Gln Arg Val Ala Leu Ala Ile Thr Pro Arg Pro Gly Ser Met Gln Leu Pro Thr Ser Pro Glu Ala Leu Thr Leu Val Arg Trp Ile Thr Arg Ser Tyr Ser Ile His Thr Gly Ala Asp Leu Phe Gly Ala Asp Ser Gln Ser Cys Gly 705 710 715 720 Gly Asp Thr Leu Leu Lys Gln Leu Trp Asp His Ser Asp Ala Ile Leu Cys Cys Ser Leu Lys Thr Asn Ala Ser Pro Val Phe Thr Phe Ala Asn Gln Ala Gly Leu Asp Met Leu Glu Thr Thr Leu Val Ala Leu Gln Asp Ile Met Leu Asp Lys Thr Leu Asp Asp Ser Gly Arg Arg Ala Leu Cys Ser Glu Phe Ala Lys Ile Met Gln Gln Gly Tyr Ala Asn Leu Pro Ala 785 790 795 800

#### MBI0018 Sequence Listing.ST25

Gly Ile Cys Val Ser Ser Met Gly Arg Pro Val Ser Tyr Glu Gln Ala 805 810 815

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tta Leu																864	
aga Arg																912	
aat Asn 305																960	
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gga Gly 385																1200	
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gta Val																1392	
gtt Val 465																1440	
tgg Trp																1488	
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Tyr His Asp Cys Pro Lys Pro Ser Ser Ile Arg Arg Gln Gln Leu Ile 35 40 45

Arg Glu Cys Pro Ile Leu Ser Asn Ile Glu Pro Lys Gln Ile Lys Val 50 55 60

Trp Phe Gln Asn Arg Arg Cys Arg Glu Lys Gln Arg Lys Glu Ala Ser 65 70 75 80

Arg Leu Gln Ala Val Asn Arg Lys Leu Thr Ala Met Asn Lys Leu Leu 85 90 95

Met Glu Glu Asn Asp Arg Leu Gln Lys Gln Val Ser Gln Leu Val His 100 105 110

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Ala Ser Gln Asn Pro Gln Arg Asp Ala Ser Pro Ala Gly Leu Leu Ser 145 150 155 160

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### MBI0018 Sequence Listing.ST25

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Gln	Leu	Tyr	Ala 260	Pro	Thr	Thr	Leu	Ala 265	Pro	Pro	Arg	Asp	Phe 270	Trp	Leu
Leu	Arg	Tyr 275	Thr	Ser	Val	Leu	Glu 280	Asp	Gly	Ser	Leu	Val 285	Val	Cys	Glu
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Cys	Asp	Gly	Gly	Gly 325	Ser	Ile	Ile	His	Ile 330	Val	Asp	His	Met	Asp 335	Leu
Glu	Ala	Cys	Ser 340	Val	Pro	Glu	Val	Leu 345	Arg	Pro	Leu	Tyr	Glu 350	Ser	Pro
Lys	Val	Leu 355	Ala	Gln	Lys	Thr	Thr 360	Met	Ala	Ala	Leu	Arg 365	Gln	Leu	Lys
Gln	Ile 370	Ala	Gln	Glu	Val	Thr 375	Gln	Thr	Asn	Ser	Ser 380	Val	Asn	Gly	Trp
Gly 385	Arg	Arg	Pro	Ala	Ala 390	Leu	Arg	Ala	Leu	Ser 395	Gln	Arg	Leu	Ser	Arg 400
Gly	Phe	Asn	Glu	Ala 405	Val	Asn	Gly	Phe	Thr 410	Asp	Glu	Gly	Trp	Ser 415	Val
Ile	Gly	Asp	Ser 420	Met	Asp	Asp	Val	Thr 425	Ile	Thr	Val	Asn	Ser 430	Ser	Pro
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Val	Ser 450	Asn	Val	Val	Leu	Cys 455	Ala	Lys	Ala	Ser	Met 460	Leu	Leu	Gln	Asn
Val 465	Pro	Pro	Ala	Ile	Leu 470	Leu	Arg	Phe	Leu	Arg 475	Glu	His	Arg	Ser	Glu 480
Trp	Ala	Asp	Asn	Asn 485	Ile	Asp	Ala	Tyr	Leu 490	Ala	Ala	Ala	Val	Lys 495	Val
Gly	Pro	Cys	Ser 500	Ala	Arg	Val	Gly	Gly 505	Phe	Gly	Gly	Gln	Val 510	Ile	Leu
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#### MBI0018 Sequence Listing.ST25

Leu Glu Gly Leu Gly His Ser Pro Glu Asp Ala Ile Val Pro Arg Asp Ile Phe Leu Leu Gln Leu Cys Ser Gly Met Asp Glu Asn Ala Val Gly Thr Cys Ala Glu Leu Ile Phe Ala Pro Ile Asp Ala Ser Phe Ala Asp Asp Ala Pro Leu Leu Pro Ser Gly Phe Arg Ile Ile Pro Leu Asp Ser Ala Lys Glu Val Ser Ser Pro Asn Arg Thr Leu Asp Leu Ala Ser Ala Leu Glu Ile Gly Ser Ala Gly Thr Lys Ala Ser Thr Asp Gln Ser Gly Asn Ser Thr Cys Ala Arg Ser Val Met Thr Ile Ala Phe Glu Phe Gly Ile Glu Ser His Met Gln Glu His Val Ala Ser Met Ala Arg Gln Tyr Val Arg Gly Ile Ile Ser Ser Val Gln Arg Val Ala Leu Ala Leu Ser Pro Ser His Ile Ser Ser Gln Val Gly Leu Arg Thr Pro Leu Gly Thr Pro Glu Ala Gln Thr Leu Ala Arg Trp Ile Cys Gln Ser Tyr Arg Gly Tyr Met Gly Val Glu Leu Leu Lys Ser Asn Ser Asp Gly Asn Glu Ser Ile Leu Lys Asn Leu Trp His His Thr Asp Ala Ile Ile Cys Cys Ser Met Lys Ala Leu Pro Val Phe Thr Phe Ala Asn Gln Ala Gly Leu Asp 740 745Met Leu Glu Thr Thr Leu Val Ala Leu Gln Asp Ile Ser Leu Glu Lys Ile Phe Asp Asp Asn Gly Arg Lys Thr Leu Cys Ser Glu Phe Pro Gln Ile Met Gln Gln Gly Phe Ala Cys Leu Gln Gly Gly Ile Cys Leu Ser Ser Met Gly Arg Pro Val Ser Tyr Glu Arg Ala Val Ala Trp Lys Val Leu Asn Glu Glu Glu Asn Ala His Cys Ile Cys Phe Val Phe Ile Asn

MBI0018 Sequence Listing.ST25 825 830

Trp Ser Phe Val 835

820

# INTERNATIONAL SEARCH REPORT

national application No.

PCT/US00/31325

IPC(7) US CL According to	SIFICATION OF SUBJECT MATTER  : C07H 21/04; C12N 5/10, 15/29, 15/63, 15/82  : 435/320.1, 419, 440, 468; 536/23.1, 23.6; 800 International Patent Classification (IPC) or to both r DS SEARCHED	0/278, 290									
Minimum do	cumentation searched (classification system followed 35/320.1, 419, 440, 468; 536/23.1, 23.6; 800/278, 2	•									
Documentation	on searched other than minimum documentation to th	e extent that such documents are included	d in the fields searched								
	ta base consulted during the international search (narontinuation Sheet	ne of data base and, where practicable, s	earch terms used)								
C. DOC	UMENTS CONSIDERED TO BE RELEVANT										
Category *	Citation of document, with indication, where ap	opropriate, of the relevant passages	Relevant to claim No.								
X	Database Genbank on NCBI, US National Library of		4,6,9,10								
	No. AJ005196, BUCHHOLZ, G. et al. 'Nuclear-lo										
Y	differentially expressed in Arabidopsis thaliana'. Se	eptember 4, 1998.	1-3,5,7,8,9,13,27-27								
X	SAKAI, H. et al. Two-component response regulators from Arabidopsis thaliana contain a putative DNA-binding motif. Plant Cell Physiology 1998, Vol 39 No. 11, pages 1232-1239, see entire document.										
Y	GLOVER, B.J. et al. Development of several epidermal cell types can be specified by the same MYB-related plant transcription factor. Development 1998, Vol 125, pages 3497-3508, see entire document.										
۸	MARTIN, C. et al. MYB transcription factors in pl 1997, Vol 13, No 2, pages 67-73, see entire docum		1-10, 13, 25-27								
Further	documents are listed in the continuation of Box C.	See patent family annex.									
	pecial categories of cited documents:	"T" later document published after the inte	rnational filing date or priority								
"A" document	defining the general state of the art which is not considered to be lar relevance	date and not in conflict with the applic principle or theory underlying the inve	ration but cited to understand the ention								
"I" carlier ap	plication or pateut published on or after the international filing date	"X" document of particular relevance; the considered novel or cannot be conside									
establish	which may throw doubts on priority claim(s) or which is cited to he publication date of another citation or other special reason (as	when the document is taken alone  "Y" document of particular relevance; the									
specified) "O" document	referring to an oral disclosure, use, exhibition or other means	considered to involve an inventive step combined with one or more other such being obvious to a person skilled in th	documents, such combination								
"P" document	published prior to the international filing date but later than the ate claimed	"&" document member of the same patent									
	ctual completion of the international search	Date of nating with 2014 itional sea	rch report								
04 April 200	1 (04.04.2001)	O Milita Care									
	ailing address of the ISA/US	Authorized officer	2.2								
	nuissioner of Patents and Trademarks PC1	David H Kruse / 69/6	W: 42								
	10.4 hington, D.C. 20231	' /									
Facsimile No	Washington, D.C. 2024 Facsimile No. (703)305-3230  Telephone No. 703-308-0196										

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31325

Box 1 Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2. Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claim Nos.: 14 and 23 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet
<ol> <li>As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</li> <li>As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite required of the control form.</li> </ol>
payment of any additional fee.  3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-10,13,25-27 and SEQ ID NO: 1,2,29&30
4. [ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

#### INTERNATIONAL SEARCH REPORT

nernational application No.

PCT/US00/31325

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING** This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXIII, claim(s) 1-10, 13, 14 and 25-27, drawn to a transgenic plant having modified structure and development characteristics, polynucleotides and vectors for producing said transgenic plant and a method of making said transgenic plant. Applicant must elect one pair of sequences (one nucleic acid and the corresponding amino acid translation) to be examined, *i.e.* SEQ ID NO: 1 and 2 in Group II, SEQ ID NO: 3 and 4 in Group II, SEQ ID NO: 5 and 6 in Group III, etc.

Group XXIV, claim(s) 11 and 12, drawn to an isolated or recombinant polypeptide.

Group XXV, claim(s) 15-17, drawn to a method of identifying a factor that is modulated by or interacts with a polypeptide.

Group XXVI, claim(s) 18, drawn to a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide.

Group XXVII, claim(s) 19 and 20, drawn to an integrated data system.

Group XXVIII, claim(s) 21-24, drawn to a method of identifying a polynucleotide or polypeptide sequence homologue.

The inventions listed as Groups I-XXVIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I-XXVIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XXIII are drawn to a transgenic plant and a method of producing said plant with a nucleic acid sequence encoding a wide variety of transcription factors. Group XXIV is drawn to a wide variety of isolated or recombinant polypeptides having transcriptional factor activity. The methods of Groups I-XXIII differ from each other in that they are directed to a plant transformation method and transgenic plant with a structurally and functionally distinct nucleic acid sequence which encodes a structurally and functionally distinct amino acid sequence. In addition, Groups XXV, XXVI, XXVIII and XXVIII are different methods from any of Groups I-XXIII in that they have different method steps and different end products, and Group XXVIII requires a computer system. Thus, there is no single special technical feature, which links the inventions of Groups I-XXVIII under PCT Rule 13.2.

Continuation of B. FIELDS SEARCHED Item 3: EAST (USPAT); STN (AGRICOLA, BIOSIS, CAPLUS, EMBASE); Sequence Search SEQ ID NO: 1, 2; 29 and 30; NCBI/Genbank.